EUROPEAN COMMISSION
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Institute for Reference Materials and Measurements

Certification of the mass fractions of crude protein, crude oils and fats, crude fibre, crude ash and phosphorus (according to method specifications laid down in EU-legislation) and of copper, calcium and magnesium

BCR-708 (synthetic dairy feed)
BCR-709 (synthetic feed for growing pigs)

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Report EUR 21070

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Institute for Reference Materials and Measurements

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SUMMARY

This report describes the preparation, homogeneity and stability studies, and the certification of nutrients analysed in the official control of nutrients in feeding stuffs. The following analytes have been studied: Crude protein (N·6.25), Crude oils and fats, Crude fibre, Crude ash, Copper, Calcium, Phosphorus and Magnesium. For the analyses comprising the "proximate analysis scheme", Crude protein, Crude oils and fats, Crude fibre and Crude ash, the studies aimed to method specific certification, according to the specifications laid down in EU-Directives. The elements on the other hand were analysed by different methods in order to produce an un-biased estimate of their respective mass fraction in the materials. Two materials have been prepared, one is a synthetic feed for dairy cows (BCR-708) and one is a synthetic feed for growing pigs (BCR-709). The certified values and their associated uncertainties are given in the tables below. Expanded uncertainties (coverage factor k=2) were expressed according to the Guide for the Expression of Uncertainties in Measurement (GUM [6]).

Certified values of mass fractions of nutrients in BCR-708 and BCR-709

Material	*BCR-708 synthetic dairy feed*	*BCR-709 synthetic pig feed*
Analyte	Mass fraction ± Uncertainty*	Mass fraction ± Uncertainty*
Crude Protein (N·6.25)	240 ± 12 g/kg	199 ± 5 g/kg
Crude Oils & Fats	65 ± 8 g/kg	51 ± 14 g/kg
Crude Fibre	93 ± 14 g/kg	56 ± 12 g/kg
Crude Ash	50.0 ± 3.0 g/kg	42 ± 4 g/kg
Calcium	4.8 ± 0.5 g/kg	1.05 ± 0.16 g/kg
Copper	37 ± 4 mg/kg	173 ± 25 mg/kg
Magnesium	1.47 ± 0.22 g/kg	1.89 ± 0.30 g/kg
Phosphorus	4.7 ± 0.4 g/kg	5.4 ± 0.7 g/kg

*Expressed as expanded uncertainties with a coverage factor k=2 according to the GUM.

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GLOSSARY

AAS	atomic absorption spectrometry
AES	atomic emission spectrometry
BCR	Community bureau of reference
CRM	certified reference material
CV	coefficient of variation
DM	dry mass
H.W.	half-width
ICP	inductively coupled plasma
k	coverage factor
RM	reference material
RT	room temperature (+20℃)
SD	standard deviation
u_{bb}	uncertainty contribution for the inhomogeneity included in U_{CRM}
u_{char}	uncertainty contribution for the batch characterisation included in U_{CRM}
u_{sts}	uncertainty contribution for the short-term stability of the material (transportation, not included in U_{CRM})
u_{lts}	uncertainty contribution for the long-term stability of the material (storage) included in U_{CRM}
U_{CRM}	expanded uncertainty of the certified value
v/v	volume per volume
$\bar{x}$	mean

1. INTRODUCTION

1.1 Background

In the official control of feeding stuffs, as well as in production and trade of feeding stuffs, chemical analyses are central tools. For several nutrients certified reference materials, for example BCR-129 (Ca, K, Mg, P, S, Zn, I, N and Kjeldahl-N) exist. This CRM is a hay powder and thus less suitable for the compound feeding stuffs, which are the main commercial feeds. Furthermore, CRMs for most of the analytes (Crude oils and fats, Crude fibre and Crude ash) in the proximate analysis scheme are not available.

1.2 Choice of the material for a CRM

The analysis of Crude protein, Crude oils and fats, Crude fibre and Crude ash, known as "*Proximate Analysis*" is the basis for the official control of nutrients in feeding stuffs. The proximate analysis consists of a series of empirical analyses, which, for legal purposes are defined by the methods described in the following Directives: 71/250/EEC (Crude ash) [1], 92/89/EEC (Crude fibre) [2], 93/28/EEC (Crude protein) [3] and 94/64/EEC (Crude oils and fats) [4]. The empirical nature of these analyses emphasises the need for relevant matrix CRMs. Since feeding stuffs for dairy cows and growing pigs differ in composition and ingredients those two types of compound feeding stuffs, referred to as "*dairy feed*" and "*pig feed*" respectively, were prepared within this project.

1.3 Design of the project

The empirical nature of several analytes, where a filtration step is a central element in the analysis of Crude fibre and Crude oils and fats introduced a problem in the design of the certification project. On one hand the requirement for any CRM to be proven homogenous, normally achieved by grinding the material finely, and on the other hand, filtration under specified conditions, and by that the risk of loosing too finely ground material, which would render the analysis useless. This led to an initial study of particle size. Thereafter, two interlaboratory comparisons where conducted, the first to establish the state of arts and familiarise the participants to the materials and a second to select the laboratories for the certification campaign.

2. PARTICIPANTS

2.1 Preparation of the materials

-	European Commission, DG JRC, Institute for Reference Materials and Measurements, Geel	BE

2.2 Homogeneity and stability testing

-	Rijksontledingslaboratorium, Tervuren	BE
-	National Veterinary Institute, Uppsala	SE
-	State Laboratory, Dublin	IE

2.3 Certification measurements

-	Laboratorio Nacional Investigaçao Veterinaria, Lisboa	PT
-	RIKILT – Institute of Food Safety, Wageningen	NL
-	Universität Hohenheim, Stuttgart	DE
-	Plantedirektoratet, Copenhagen	DK
-	Instituto National de Engenharia e Tecnologias Industrial Departemento de Tecnologias das Industrias Alimentares, INETI-DTIA, Lisboa	PT
-	Rijksontledingslaboratorium, Tervuren	BE
-	Istituto Zooprofilatico Sperimentale, Firenze	IT
-	National Veterinary Institute, Uppsala	SE
-	State Laboratory, Dublin	IE

3. Preliminary Studies

3.1 Particle size study

A basic demand for every CRM is that it is homogenous. This is, for particulate materials, normally achieved by grinding the material to a very fine powder. In this project, where the aim was to certify the levels of nutrients in compound feeding stuffs, this presented a problem. Crude fibre and crude oils and fats rely on filtration steps with defined conditions and there was a risk to loose material in the analyses by the use of too finely ground material. To determine the optimal particle size for the CRMs a preliminary study with three different particle sizes was performed. It was judged that the two most crucial analytes was crude fibre and copper, the latter commonly being added to pig feed in the form of copper sulphate, while only naturally abundant copper is found in dairy feed. From the two materials (referred to as "*dairy*" and "*pig*") five bottles each of three different particle sizes coded as "*A*", "*B*", and "*C*" were analysed for content of crude fibre and copper by two laboratories. The characteristics of the three particle sizes are presented in Table 3.1. Figures 3.1 and 3.2 present box-whisker plots of the results. No significant difference in copper or crude protein content was found. It was decided that particle size "*B*" should be used for the coming certification.

Table 3.1 - Sieve analysis of dairy and pig feed ground to different particle sizes.

Material	*Dairy feed*			*Pig feed*		
Batch identification	*A*	*B*	*C*	*A*	*B*	*C*
	Sieve fraction (% by weight)			*Sieve fraction (% by weight)*		
< 63µm	0.8	5	18	3	42	28
< 125µm	34	65	70	42	73	70
< 250µm	66	80	94	71	94	92
< 500µm	100	100	100	100	100	100

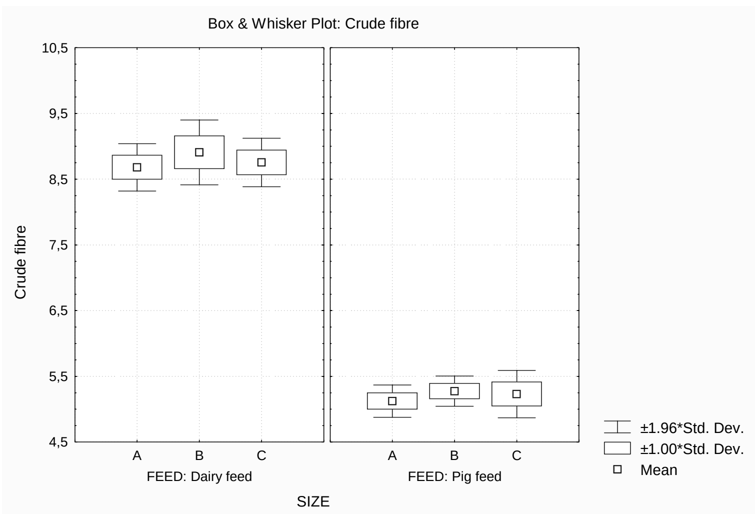

Figure 3.1 - Box-whisker plot of the content of crude fibre in the dairy and pig feeds ground to different particle sizes.

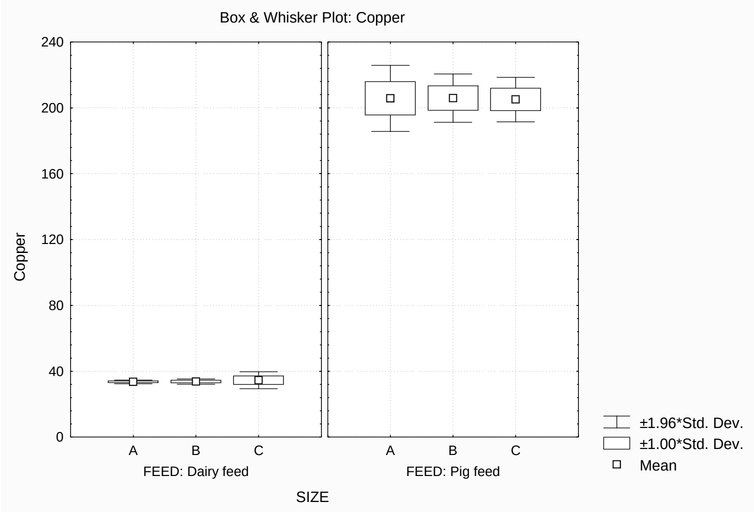

Figure 3.2 - Box-whisker plot of the Cu-content in dairy and pig feeds at different particle sizes.

3.2 Familiarisation study (Intercomparison 1)

The two materials ("*dairy B*", and "*pig B*") were used in a preliminary Ring-test. 18 laboratories attended the study. Two sets of samples, consisting of 3 bottles, were sent to each laboratory. The labs were instructed to analyse the samples in 5 replicates on two separated days (3+2 determinations). It was stressed that all proximate analyses should be made with the exact procedure as written in the respective Directive. The result from the first Interlaboratory comparison is presented in Table 3.2.

Table 3.2 - Summary of results analysis (Mean of means ± 95 % confidence interval) of dairy and pig feeds "B" from Interlaboratory comparison 1.

	Crude protein (g/kg)	*Crude oils & fats (g/kg)*	*Crude fibre (g/kg)*	*Crude ash (g/kg)*
Dairy feed	221.47 ± 2.95	64.51 ± 1.49	86.81 ± 2.02	46.69 ± 0.31
Pig feed	183.2 ± 1.02	45.69 ± 1.83	50.13 ± 1.06	38.03 ± 0.46
	Copper (mg/kg)	*Calcium (g/kg)*	*Phosphorus (g/kg)*	*Magnesium (g/kg)*
Dairy feed	29.43 ± 4.49	5.30 ± 0.50	4.36 ± 0.07	1.40 ± 0.07
Pig feed	205.3 ± 10.26	1.32 ± 0.90	5.07 ± 0.075	1.86 ± 0.054

3.3 Selection of certification laboratories (Intercomparison 2)

A second round of Interlaboratory comparisons was performed using one material. This test material was a mixture of the candidate reference materials (see below for details). From the result of this interlaboratory comparison laboratories were selected for the certification analyses.

Table 3.3 - Summary of results of analyses in Interlaboratory comparison 2.

	Crude protein (g/kg)	*Crude oils & fats (g/kg)*	*Crude fibre (g/kg)*	*Crude ash (g/kg)*
Mean of means	203.9 ± 1.40	55.5 ± 1.19	68.7 ± 2.60	42.8 ± 0.36
	Copper (mg/kg)	*Calcium (g/kg)*	*Phosphorus (g/kg)*	*Magnesium (g/kg)*
Mean of means	106.9 ± 5.60	2.58 ± 0.29	4.91 ± 0.14	1.65 ± 0.046

4. PREPARATIONS OF THE MATERIALS

4.1 Preparation of the powder

The preparation of all test materials as well as the materials in the candidate CRMs was done at the IRMM in Geel, Belgium. Feed ingredients were obtained locally from AVEVE VEEVOEDING, Merksem, Belgium, by assistance from Rijksontledingslaboratorium in Tervuren, Belgium. A synthetic feed for dairy cows (BCR-708) and a synthetic feed for growing pigs (BCR-709) were formulated. Compositions of the candidate CRMs are presented in Tables 4.1 and 4.2, respectively. The ingredients for the respective materials were chosen to include relevant base materials used in contemporary commercial feeding stuffs. One important difference between the two RMs is that copper was added to the "*pig feed*" (BCR-709), while the copper in the "*dairy feed*" (BCR-708) was naturally abundant. Copper is used as a feed additive in pig feed but ruminants, especially sheep, are susceptible to copper poisoning.

Table 4.1 - Composition of "dairy feed", BCR-708.

Ingredient	*(%)*
Maize gluten meal	8
Dried brewer's grain	30
Barley	22
Citrus pulp	20
Soy bean	12
Rapeseed meal	3
Dairy mineral pre-mix*	5
Total	100

*Composition: 7.4 % dairy minerals and 92.6 % soy bean meal.

Table 4.2 - Composition of "dairy feed", BCR-709.

Ingredient	*(%)*
Wheat	25
Barley	25
Maize gluten feed	25
Soy beans	10
Peas	10
Pig mineral pre-mix*	5
Total	100

*Composition: 7.4 % pig minerals and 92.6 % soy bean meal.

The coarse ingredients: wheat, barley, brewer's grain, citrus pulp, peas and soybeans were pre-crushed in 160 UPZ mill (Alpine Co.) The finer materials: maize gluten meal, maize gluten feed, and rape seed meal were not pre-crushed. Special concern was taken to the added minerals since it could be foreseen that inhomogeneity in the mixing and separation of mineral particles by sedimentation in the prepared materials could occur. Therefore, a premix of minerals and soy bean powder was made using a suspension technique. Minerals and soy bean powder was mixed with de-mineralised water in an Ultra Turrax and subsequently freeze-dried. This procedure has been shown to enhance the homogeneity with regard to minerals. All ingredients were ground in an 100 UPZ mill (Alpine Co.) fitted with a 0.5 mm stainless steel sieve and the two materials were prepared by turbula mixing according to Table 4.1 and 4.2, respectively. The resulting materials were freeze-dried. The total amount of each material was about 160 kg and thus the mixing was performed in four batches of 40 kg each for the two materials.

Table 4.3 - Sieve analysis of the synthetic feeds BCR-708 "Dairy", BCR-709 "Pig" and the test mixture used for Interlaboratory comparison II.

Sieve fraction (% by weight)	*BCR-708*	*BCR-709*	*Mixture 1*
<90 µm	37	41.6	49.3
<125 µm	49.2	53	60
<180 µm	62.6	64.2	71.7
<250 µm	76.6	77.0	84.0
<355 µm	92.4	92.4	96.3
<500 µm	100	100	100

4.2 Bottling and packaging

The freeze-dried materials were filled under argon in 100 ml amber glass bottles with polyethylene inserts to contain 40 g per bottle. During bottling equal amounts of the four batches from turbula mixing were homogenised in order to eliminate any possible between batch inhomogeneity. In all 3422 bottles of BCR-708 and 3725 bottles of BCR-709 were produced in April 2000. All test materials used for the preliminary studies were prepared in the same way and using the same base materials (ingredients) as the final reference materials (BCR-708 and BCR-709). The test material for Interlaboratory comparison 2 was a 40/60 % (w/w) mixture of BCR-708 and BCR-709. The composition was, however, unknown to the participants of the Interlaboratory comparison.

4.3 Gamma irradiation

In order to prevent microbial growth the materials BCR-708 and BCR-709 were gamma-irradiated at a dose of 5 kGy (Gammaster BV, Etten Leur, NL).

5. TESTING OF THE MATERIALS

5.1 Homogeneity tests

5.1.1 Design of the study

Homogeneity of the two materials was checked by analyses of “*crude protein*”, a main component and copper, the only “*micro-mineral*” to be certified. Furthermore, as copper was added to the “*pig*” feed, assessment of this element was of special interest. 40 bottles, taken as “*stratified random samples*” of each material were analysed in duplicate (in random order) at the State Laboratory in Dublin, Ireland.

5.1.2 Method used

Nitrogen was determined with a combustion method (Dumas method) and the results were expressed in the terms of crude protein by multiplication by 6.25 in order to get a similar order of magnitude of the figures.

Copper was analysed by ICP-AES after dry-ashing.

5.1.3 Results

The results of the homogeneity study are presented in Figures 5.1 to 5.4.

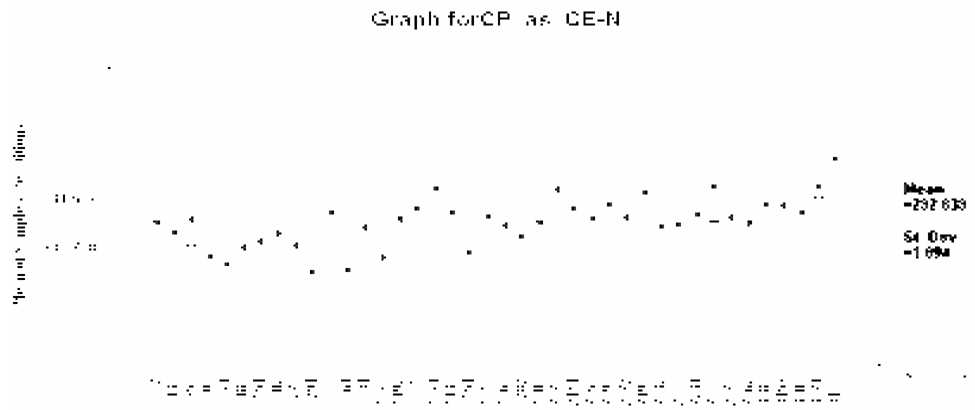

Figure 5.1 - Content of “crude protein” (as 6.25·N determined by the “Dumas method”) in different bottles of candidate BCR-708.

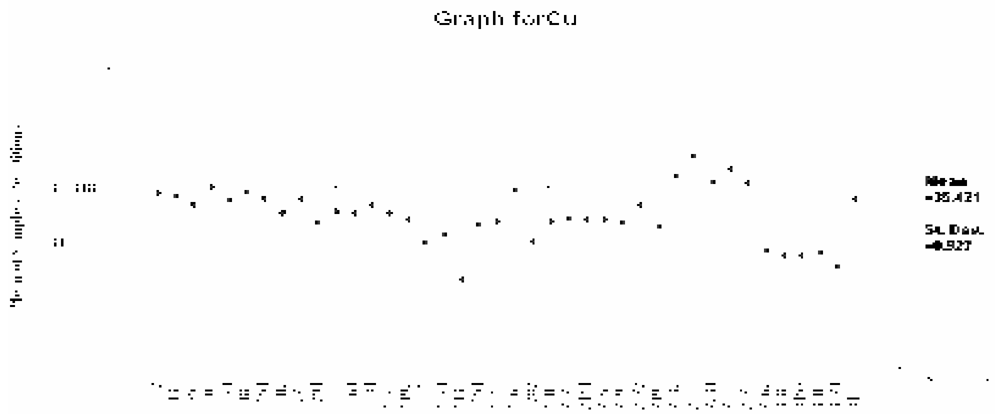

Figure 5.2 - Content of copper in different bottles of candidate BCR-708

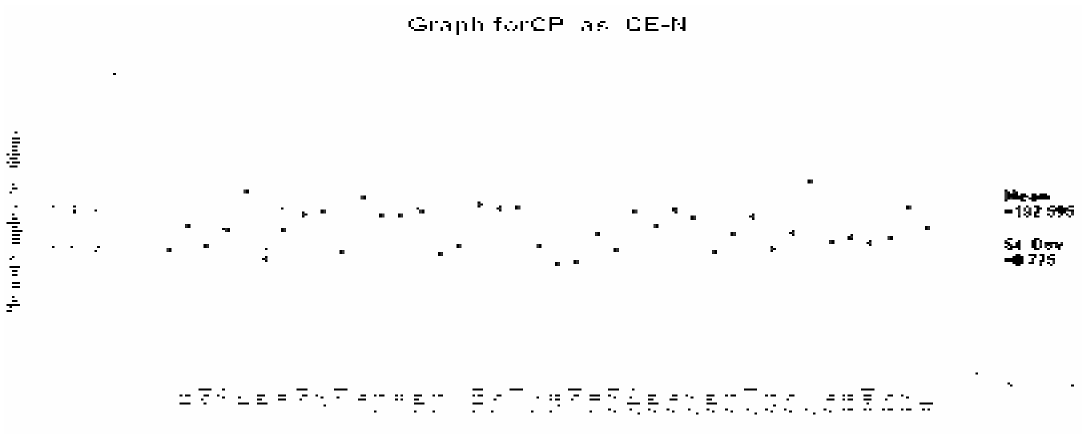

Figure 5.3 - Content of “crude protein” (as 6.25·N determined by the “Dumas method”) in different bottles of candidate BCR-709.

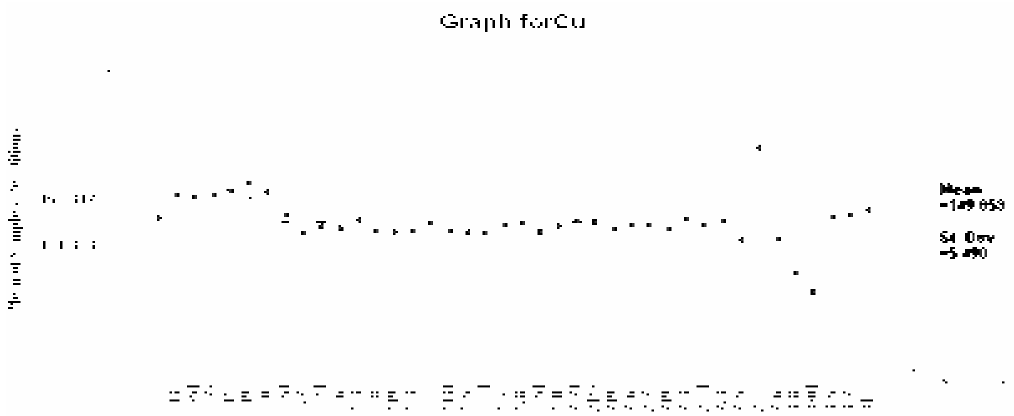

Figure 5.4 - Content of copper in different bottles of candidate BCR-709

5.1.4 Conclusion

It was concluded that no systematic variation in contents of nitrogen or copper was present in the two materials. 2-3 unexpectedly high differences between duplicates (from the same bottles) were present as can be seen from the figures above. Although the homogeneity study was carried before the requirements of ISO Guide 34 [5] and the GUM [6] could be integrated into the project, an uncertainty contribution from the homogeneity study was incorporated into the expanded uncertainty. For further details refer to section 7.2.

5.2 Stability test

5.2.1 Design of the study

Stability testing of the two materials (BCR-708 and BCR-709) was performed by storage of the materials at –20 °C (reference temperature), +4 °C, +20 °C, +40 °C and at +70 °C for 8 weeks with sampling every 2 weeks. The highest temperature, +70 °C, was used for an accelerated stability test. The materials are highly unlikely to be exposed to this temperature but the inclusion of this step was made to provoke deterioration and give a quick indication of stability for longer storage. Samples stored at +4 °C and +20 °C were also sampled after 52 weeks of storage. Crude protein, crude oils and fats, crude fibre, crude ash, Ca, P and Mg were analysed at each point in time. Two bottles per point in time were analysed (single determinations) and the mean value was used for evaluation.

Supportive stability data were supplied by the analysis of samples from the preliminary particle size study (Particle size "*C*") stored at –20 °C (reference temperature), +4 °C, +20 °C for 48 months. Those samples were analysed in triplicate.

5.2.2 Method used

Methods of analyses for the proximates were the same as used in the certification except for crude oils and fats, which were determined by hot extraction using FOSS-Tecator Soxtec (FOSS AB, Höganäs Sweden) equipment.

5.2.3 Results

The results of the stability trials are presented in Annex III. For all temperature series and all analytes except for crude fibre stored at +20 °C determined in BCR-708, the regression lines (R_T) were not significantly different from zero, indicating that differences in the measurements not were due to alteration of the samples by time/temperature of storage. The determination of crude fibre in BCR-708 stored at +20° C was disturbed probably due to some handling errors on the sample. For BCR-709, however, the regression (R_T) differed from zero in two cases. i) For crude ash stored at +70 °C, where it was noted that the plastic seal of the bottles were damaged, probably by heat, and thus exposing the material to (relatively) hot air; and ii) for magnesium stored at +20 °C, where the last point, by unknown reason was significantly higher than the others.
From the analyses of samples from the study of test material "*C*" stored at –20 °C (reference temperature), +4 °C, +20 °C it was observed that Crude Oils and Fats might be at risk of deterioration during storage at temperatures over the reference temperature. This observation, however, was noted only for the synthetic pig feed and the data is based on analyses of the contents of only one bottle. It is, however, important to pay special attention on the content of Crude Oils and Fats during the stability monitoring of BCR-708 and 709.

5.2.4 Conclusions

The stability study indicates that the materials are rather robust with respect to the analytes of interest when kept in unopened bottles. This should make storage and handling inexpensive.

Similar as in case of the homogeneity study, stability testing was carried before the requirements of ISO Guide 34 [5] and the GUM [6] could be integrated into the project. To achieve a GUM-compliant uncertainty statement, the data of Annex III were used to plot a provisional shelf-life and to include an additional uncertainty contribution into the expanded uncertainty. For further details refer to section 7.2. Parallel to this stability monitoring will be continued using isochronous testing schemes as proposed by Lamberty *et al.* [7].

5.3 Storage and transport

The materials should be stored in non-condensing atmosphere. Refrigerated transport is not necessary.

6. CERTIFICATION MEASUREMENTS

6.1 Analytical methods used

In this project four operationally defined analytes (proximate analysis) and four elements have been studied. In order to assure the strict application of the defined procedures for the proximate analyses questionnaires were sent to all participants in the certification exercise. The participants were asked to declare known deviations, or, in the case of less explicitly defined procedures in the Directives, the actual procedure used at each laboratory. The information from these questionnaires is tabulated for each analyte in Annex I (tables 1-8, respectively). For the elements, copper, calcium, magnesium and phosphorus, i.e. the non-empirical analytes, the participants were asked to use their methods of preference. Also the labs were responsible for calibration of their own instruments. No common calibrants were recommended or distributed.

Crude protein is in this context defined by the procedure laid down in Directive 93/28/EEC [[3]]. That is, nitrogen is determined by the Kjeldahl-method and crude protein is the amount of nitrogen multiplied by the factor 6.25. Due to technical reasons, several laboratories were unable to use the prescribed end-determination, i.e. using sulphuric acid as receiving solution and titration with sodium hydroxide, but have used boric acid as receiving solution and hydrochloric acid for titration. This deviation from procedure did not seem to give a systematic difference in the results. It was thus decided not to exclude laboratories from the certification campaign on that ground.

Crude oils and fats are defined by Directive 94/64/EEC [[4]]. This is a slightly more complicated analyte because the directive contains two general procedures, "*A*" and "*B*", where in the latter a hydrolysis step precedes the extraction. In this project, only method B has been considered. Method "*B*" is the more general approach and in cases where different yields of crude oils and fats are obtained when using methods "*A*" and "*B*", the highest yield is normally associated with method "*B*". Thus it is stated in the Directive that if a higher yield is obtained using method "*B*", this should be the valid result. The hydrolysis step can be done manually with filter-paper, this procedure is quite laborious and involves material transfer that might cause reproducibility problems. On the market there exists also a semi-automatic apparatus for the hydrolysis, which reduces the amount of manipulation with the material. Theoretically, this should improve the analytical precision. In addition, the Directive allows two different extraction procedures, Soxhlet extraction and continuous extraction. Only one laboratory has used the continuous procedure.

Crude fibre is defined in Directive 92/89/EEC [[2]]. This method is in its present state well adapted for semi-automated extraction and filtration using crucibles with sintered glass filters. Formerly a manual filtration step using asbestos or glass-wool was utilised but from environmental reasons asbestos has been banned from use. One crucial point is the condition of the sintered glass crucibles which may alter the filtration characteristics by ageing.

Crude ash is defined by the procedure in Directive 71/250/EEC [[1]]. In the Directive the demands on temperature control are high, 550 $\pm$ 5 °C. In reality this precision is very hard to achieve. It is also stated that the samples should be carbonised on a hot plate prior to the ashing in the muffle oven. An alternative has been presented where this step is omitted and replaced with putting the samples directly into the cold muffle oven and keeping them there during the heating of the oven.

Copper, calcium and magnesium were determined by either ICP-AES or AAS after dry- ashing and dissolution in dilute HCl except for one laboratory who used wet-digestion in closed vessels (microwave heating in teflon bombs).

Phosphorus was determined by ICP-AES or by UV-spectrophotometry after treatment by vanadate-molybdate-reagent as described in Directive 71/393/EEC [8]. Dry-ashing was the predominant sample preparation step.

6.2 Presentation of the results

The summary of analytical results including outcome of statistical tests is presented in Tables 7.1 (BCR-708) and 7.2 (BCR-709). The results of individual measurements are presented in Annex II.

6.3 Technical discussion

6.3.1 Agreement between methods/laboratories

All data from the certification analyses were discussed in detail at an evaluation meeting were all participating labs attended. Extensive discussions were held regarding all methods used. A thorough discussion on good analytical control and quality control in general was held at the technical meeting. Special care was taken to trace possible modifications of the methods for the method-defined (empirical) analytes. This was of particular interest since the different methods are written to various degree of detail in the Directives in question. The empirical methods are all associated with potential problems of mainly two kinds. i) Procedures might have to be changed from non-analytical reasons, such as environmentally or health related risks (e.g. asbestos in the crude fibre method), and ii) the strict defined procedures might hinder technical progress (e.g. extraction conditions in the analysis of crude fats and oils). Another problem with the empirical method is the impossibility of using calibrants.

6.4 Acceptance of data

No data was rejected from statistical reason based on a single statistical test. Successive use of the same test e.g. Cochran's test for outlying variances was not the basis to exclude laboratories. Data were excluded from statistical reasons on the basis of several tests simultaneously indicating outliers. In the case of the analysis of crude ash one data-point from BCR-708 and one from BCR-709 were rejected due to clearly revealed technical reason. For BCR-708 one data point was lost in the analysis of crude fats and oil due to an error at the laboratory.

7. EVALUATION, CERTIFIED VALUES AND UNCERTAINTIES

7.1 Technical discussion of data

The statistical evaluation was done according to the "*BCR-Guidelines*" [9]. All calculations in the certification campaign were done using the SoftCRM v.1.1.0 software package [10].

Pooling of the data was not allowed according to the prescribed tests Snedecor F-test and Bartlett's test. Thus the certified values are in all cases based on the calculation of mean of means.

As can be seen in Table 7.1 and 7.2 the populations of results used as the basis for certified values had normal distributions, as determined by the Kolmogorov-Smirnov-Lilliefors tests, for all analytes except for copper in BCR-708. In this case the content of copper is in the low range of the methods used for the determination of copper in feed. This fact may contribute to the observed effect.

For the empirical analytes (the proximates), crude protein, crude oils and fats, crude fibre and crude ash, all laboratories were required to use the same method. This would imply that pooling of the data should be possible. Nevertheless, the statistical evaluation did not allow this procedure. The reasons for the significant differences between laboratories are not known. One might speculate that these methods, being very old, involving many manual moments, and traditional, have developed slightly different in different parts of Europe. The absence of CRMs or pure calibrants is a notable problem. One way of judging laboratory skill and conformity within the community of laboratories is to study the results from international proficiency testing programmes. A summary of such data are provided in Annex I.

Table 7.1 - Summary of statistical results – BCR-708 – A synthetic feed for dairy cows.

Certified property	*Crude protein*	*Crude oils & fats*	*Crude fibre*	*Crude ash*	*Cu*	*Ca*	*Mg*	*P*
Number of data sets	6	7	9	8	6	7	8	6
Number of individual data	36	41	54	47	36	42	48	36
Compatibility of data sets 2 by 2 (Scheffe's)	No	No	No	No	No	No	No	No
Outlying data sets (Dixon. Nalimov)	No	No	No	Yes	Yes	Yes	No	No
Outlying variances (Cochran)	No	Yes	No	No	Yes	No	No	Yes
Mean of means of data sets (g/kg)	239.596	64.66	93.297	50.017	37.136 *	4.795	1.474	4.748
Within lab standard deviation	1.029	1.057	1.273	0.439	1.434 *	0.093	0.046	0.099
Between lab standard deviation	1.059	1.603	3.645	0.186	0.870*	0.058	0.041	0.046
Homogemeity of variance (Bartlett)	Yes	Yes	No	No	N/A	N/A	N/A	N/A
Standard deviation of data set means (g/kg)	1.040	1.666	3.682	0.257	1.049 *	0.069	0.045	0.061
Normality of the distribution of data set means (Kolmogorov-Smirnov-Lilliefors)	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
Half width of 95 % confidence interval (g/kg)	1.196	0.580	2.830	0.215	1.101 *	0.064	0.038	0.064

Table 7.2 - Summary of statistical results – BCR-709 – A synthetic feed for growing pigs.

Certified property	*Crude protein*	*Crude oils & fats*	*Crude fibre*	*Crude ash*	*Cu*	*Ca*	*Mg*	*P*
Number of data sets	7	6	8	9	6	7	8	6
Number of individual data	42	36	48	53	36	42	48	36
Compatibility of data sets 2 by 2 (Scheffe's)	No	No	No	No	No	No	No	No
Outlying data sets (Dixon. Nalimov)	Yes	No	Yes	No	Yes	No	No	No
Outlying variances (Cochran)	Yes	No	No	No	Yes	Yes	Yes	Yes
Mean of means of data sets (g/kg)	198.789	50.584	56.209	41.813	173.288*	1.051	1.891	5.412
Within lab standard deviation	1.011	0.948	1.066	0.314	3.756*	0.048	0.054	0.103
Between lab standard deviation	1.823	1.293	1.016	0.311	4.605*	0.077	0.068	0.029
Homogemeity of variance (Bartlett)	No	No	Yes	Yes	N/A	N/A	N/A	N/A
Standard deviation of data set means (g/kg)	1.869	1.349	1.106	0.333	4.854*	0.078	0.072	0.051
Normality of the distribution of data set means (Kolmogorov-Smirnov-Lilliefors)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Half width of 95 % confidence interval (g/kg)	1.729	1.416	0.924	0.256	5.094*	0.073	0.060	0.053

7.2 Uncertainty evaluation

The evaluation of uncertainties in the context of certification exercises has evolved over the past decade. Nowadays, certified values should be accompanied by uncertainty statements in compliance with the requirements made by GUM [[6]]. While the design of new certification projects consider the needs for a proper estimation of the various uncertainty sources such as stability and homogeneity, older campaigns aimed only on qualitative statements (yes/no decisions) whether a material was stable and homogeneous.

The evaluation described hereafter is based on a concept described by Pauwels *et al.* [11] and literature cited] and uses available data discussed in the previous chapters.

7.2.1 Introduction and statistical concept

The combined (and expanded) standard uncertainty on a reference material should consider that in addition to the characterisation of the batch, homogeneity, and long- and short-term stability play an important role. Therefore, the uncertainty can be expressed as:

- Uncertainty of the certified value as obtained for the batch (characterisation, u_{char});
- Transferred to a single package (homogeneity, u_{bb});
- As dispatch to the customer (short-term stability, u_{sts});
- At the time of sale (long-term stability, u_{lts}).

Following this and based on the data obtained in the stability and homogeneity studies as well as the results of the batch characterisation, estimates for u_{bb} (homogeneity), u_{lts} (long-term-stability) and u_{char} (batch characterisation) were obtained and combined according the following equation [11 and literature cited]:

$$U_{CRM} = 2 \cdot \sqrt{u_{bb}^2 + u_{lts}^2 + u_{char}^2}$$

Due to the transport conditions selected for dispatch, the uncertainty constituent for short-term stabililty (u_{sts}) is negligible and consequently not included in the overall uncertainty. The estimation of the other uncertainty sources is described below.

7.2.2 Uncertainty source "homogeneity"

The homogeneity study is exhaustively described in chapter 5.1. Unfortunately, the results do not allow to separate between variations due to the residual heterogeneity of the material and those due to the measurement method applied. Therefore, the standard deviations expressed in Figures 5.1 to 5.2 were used as a conservative estimate of u_{bb}.

As homogeneity was tested only for parameters "*Crude Proteins*" and "*Cu*", the larger value of both was used as Type-b estimate according to the GUM [6] of the u_{bb} of the other certified property values.

7.2.3 Uncertainty source "stability"

The stability data discussed in chapter 5.2 may appear qualitatively sufficient to deem the material to be stable. A quantitative estimation of this stability, i.e. u_{lts}, was obtained by plotting a shelf-life using the data of 4℃ as reference temperature and those of 20℃ as normal storage temper ature (see Annex III). As the number of points is rather limited, the plot was limited to a period of 2 years.

The plotting was done according the principles described elsewhere [12].

7.2.4 Uncertainty source "batch characterisation"

An estimate for u_{char} was derived from the standard error obtained on the mean of laboratories means.

7.2.5 Uncertainty budget

Based on the uncertainty contributions mentioned in sections above the following uncertainty budgets are established:

Table 7.3– Uncertainty budget and certified values for BCR-708

Parameter	*Mean value*	u_{char} *(%)*	u_{bb} *(%)*	u_{lts} *(%) 24 months*	U_{CRM} *(%)*	U_{CRM}	*Certified value*	*Unit*
Crude Protein	239.596	0.18	0.81	2.35	4.98	11.94	240 ± 12	g/kg
Crude Fats	64.66	0.97	2.62	5.05	11.54	7.46	65 ± 8	g/kg
Crude fibre	93.297	1.32	2.62	6.4	14.08	13.14	93 ± 14	g/kg
Crude ash	50.017	0.18	2.62	1.4	5.95	2.98	50.0 ± 3.0	g/kg
Ca	4.795	0.54	2.62	4.15	9.87	0.47	4.8 ± 0.5	g/kg
Cu	37.136	1.15	2.62	4.15	10.08	3.74	37 ± 4	mg/kg
P	4.748	0.52	2.62	3.19	8.32	0.40	4.7 ± 0.4	g/kg
Mg	1.474	1.08	2.62	6.73	14.60	0.22	1.47 ± 0.22	g/kg

Table 7.4 – Uncertainty budget and certified values for BCR-709

Parameter	*Mean value*	u_{char} *(%)*	u_{bb} *(%)*	u_{lts} *(%) 24 months*	U_{CRM} *(%)*	U_{CRM}	*Certified value*	*Unit*
Crude Protein	198.789	0.36	0.4	1.01	2.29	4.55	199 ± 5	g/kg
Crude Fats	50.584	1.09	3.66	13.08	27.25	13.79	51 ± 14	g/kg
Crude fibre	56.209	0.7	3.66	9.34	20.11	11.30	56 ± 12	g/kg
Crude ash	41.813	0.27	3.66	2.64	9.04	3.78	42 ± 4	g/kg
Ca	1.051	2.81	3.66	5.77	14.78	0.16	1.05 ± 0.16	g/kg
Cu	173.288	1.14	3.66	5.77	13.85	24.01	173 ± 25	mg/kg
Mg	1.891	1.35	3.66	6.76	15.61	0.30	1.89 ± 0.30	g/kg
P	5.412	0.38	3.66	4.94	12.32	0.67	5.4 ± 0.7	g/kg

7.3 Certified values

The certified values (unweighed mean of the accepted sets of results) and their uncertainties (combined uncertainty with a coverage factor of k=2) are summarised in table 7.6. Values are rounded according to ISO Standard 31-0 [[13]].

Table 7.5 - Certified values of mass fractions of nutrients in BCR-708 and BCR-709

Analyte	*BCR-708 a synthetic dairy feed* *Mass fraction ± Uncertainty**	*BCR-709 a synthetic pig feed* *Mass fraction ± Uncertainty**
Crude Protein (N·6.25)	240 ± 12 g/kg	199 ± 5 g/kg
Crude Oils & Fats	65 ± 8 g/kg	51 ± 14 g/kg
Crude Fibre	93 ± 14 g/kg	56 ± 12 g/kg
Crude Ash	50.0 ± 3.0 g/kg	42 ± 4 g/kg
Calcium	4.8 ± 0.5 g/kg	1.05 ± 0.16 g/kg
Copper	37 ± 4 mg/kg	173 ± 25 mg/kg
Magnesium	1.47 ± 0.22 g/kg	1.89 ± 0.30 g/kg
Phosphorus	4.7 ± 0.4 g/kg	5.4 ± 0.7 g/kg

*Expressed as expanded uncertainties with a coverage factor k=2 according to the GUM.

8. INSTRUCTIONS FOR USE

8.1 Sample handling

The material is packed under argon in amber glass bottles. Each bottle contains about
40 g of material. The material is freeze-dried and contains normally less than 6 % moisture. The certified quantities are only valid for use directly after opening of the bottle. The moisture content must be analysed by oven drying at 103 ± 2 °C for 5 h in direct conjunction to analysis of the certified analytes.

No special risk is associated with the handling or the use of the materials. The materials should be stored in the dark under a non-condensing atmosphere. Before opening, the bottle should be shaken gently for one minute. After opening, the material should be stirred carefully and subsamples for the individual analyses should be taken at the same occasion in order to ensure that these sub-samples are homogenous and the material is unaltered by exposure to normal atmosphere.

Opened bottles should be stored in a desiccator over s suitable drying agent. It should be stressed that the material is finely ground and it is crucial to use a filtering aid (celite) in the analyses of crude fibre ad crude oils and fats.

8.2 Use of the CRMs in quality control

These materials can be used for control of analytical performance if the user can prove repeatability of the method in question. The laboratory bias may be estimated by taking the difference between the mean values of replicate laboratory measurements (X) and the certified value (μ): X-μ.

The criterion for acceptance is given in ISO Guide 33 [[14]] as follows:

$$a_2 - 2s_L < X - \mu < a_1 + 2s_L \text{ where}$$

a_1 and a_2 are the adjusted values, chosen by the user according to economic or technical limitations or stipulations and s_L is the long-term within-laboratory standard deviation.

9. References

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[13] ISO (1992). *ISO-Standard 31 - Quantities and units – Part 0: General principles*. Geneva, Switzerland.

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10. ANNEX I - COMPILATION OF METHODS

Table 10.1 - Determination of crude protein and deviations from Directive 93/28/EEC.

Lab no	*Sample intake*	*Catalyst*	*Collection liquid*	*Indicator*	*Method control*	*Recovery*
Dir	1g	$CuO/CuSO_4$	H_2SO_4	Methyl red	Acetanilide	>99 %
0		Kjeltabs Cu/3.5	H_3BO_3		$(NH_4)_2$ SO_4-, CRM, Acetanilide	
1		Kjeltabs Cu/3.5				99.52
2		K_2SO_4 Se	H_3BO_3 4 % (m/V)	Buchi system		
3		Special tablets from Gerhardt: 5 g K_2SO_4 / 0,5 g $CuSO_4x5H_2O$			$(NH_4)_2$ SO_4-solution and a feedstuff-sample (out of a ringtest)	
4						
5						
6		Kjeltabs :2* 5 g K_2SO_4 / 0,5 g $CuSO_4x5H_2O$ 1·5 g K_2SO_4 / 0,1 g $CuSO_4x5H_2O$		Buchi system		
7		$CuSO_{4..}$ 5 H_2O				Day 1: 99.3 % Day 2: 99.1 %
8		K_2SO_4 $CuSO_4{\cdot}5H_2O$	Boric Acid 1 %	Bromocresolgreen Methyl red $H_3SO_4{\cdot}{\cdot}0.2N$		

Table 10.2 - Determination of crude oils and fats and deviations from Directive 94/64/EEC.

Lab no	*Sample intake*	*Extraction principle*	*Hydrolysis system*	*Filter aid*	*Drying of extract*
Dir	2.5 g	Soxhlet or Continuous	Manual	e.g. Kieselguhr	100℃ / 90 min
0		Continuos	Semi-automatic		
1		Soxhlet	Manual	Kieselguhr	
2		Soxhlet		Not used	
3		Soxhlet			100℃ / 60 min
4			Semi-automatic		
5					
6		Soxhlet		Diatomaceous Earth	
7		Soxhlet		Not used	
8		Soxhlet			90 minutes + 30minutes

Table 10.3 - Determination of crude ash and deviations from Directive 71/250/EEC.

Lab no	*Sample intake*	*Crucible type*	*Pre-treatment*	*Temp/time*
Dir	5 g	Pt; Pt/Au	Hot plate	Until free from organics
0		Ceramic	Cold oven	2h
1		Ceramic		24h
2		Quartz		12-16h
3		Porcelain		3h
4				
5				
6		Silica		4h
7				4h
8				>3h

Table 10.4 - Determination of crude fibre and deviations from Directive 92/89/EEC.

Lab no	*Sample intake*	*Crucibles (old/new)*
Dir	1 g	N/A
0		O
1		N
2		N
3		N
4		N
5		O
6		O
7		O
8		O

Table 10.5 - Methods of analysis for the determination of copper.

Lab no	*Sample intake*	*Dry ashing*	*Wet digestion*	*ICP*	*AAS*
0		X		X	
1		X			X
2		X		X	
3		X			X
4		X			X
5		X			X
7	1 g		Closed		X
8		X		X	

Table 10.6 - Methods of analysis for the determination of calcium.

Lab no	*Sample intake*	*Dry ashing*	*Wet digestion*	*ICP*	*AAS*
0			Open	X	
1		X			X
2		X		X	
3		X			X
4		X			X
5		X			X
7	1 g		Closed		X
8		X		X	

Table 10.7 - Methods of analysis for the determination of magnesium.

Lab no	*Sample intake*	*Dry ashing*	*Wet digestion*	*ICP*	*AAS*
0			Open	X	
1		X			X
2		X		X	
3		X			X
4		X			X
5		X			X
7	1 g		Closed	X	
8		X		X	

Table 10.8 - Methods of analysis for the determination of phosphorus

Lab no	*Sample intake*	*Dry ashing*	*Wet digestion*	*ICP*	*AAS*	*Spectrophotmetr y*
0			Open	X		
1		X				X
2		X		X		
3		X				X
4		X				X
5		X				X
7			Closed	X		X
8		X		X		

11. Annex II – Individual Data

Table 11.1 – BCR-708 Content of crude protein.

Lab	Mean	Std. Dev.	H.W. CI (95 %)	Sample #1	Sample #2	Sample #3	Sample #4	Sample #5	Sample #6
1	238.750	0.630	0.661	237.890	239.250	238.310	239.330	239.330	238.390
2	239.068	0.659	0.692	238.380	239.450	239.500	239.950	238.770	238.360
3	238.513	1.496	1.570	235.840	237.800	238.740	239.570	239.840	239.290
5	241.630	0.536	0.563	242.050	242.260	241.270	241.800	240.790	241.610
7	239.583	0.776	0.814	240.300	240.700	239.600	238.700	239.100	239.100
8	240.033	1.548	1.624	237.390	239.500	241.880	240.800	240.840	239.790
			Range [min..max]						[235.840 .. 242.260]
			Mean of means						239.596
			95 % H.W. Confidence Interval						1.196

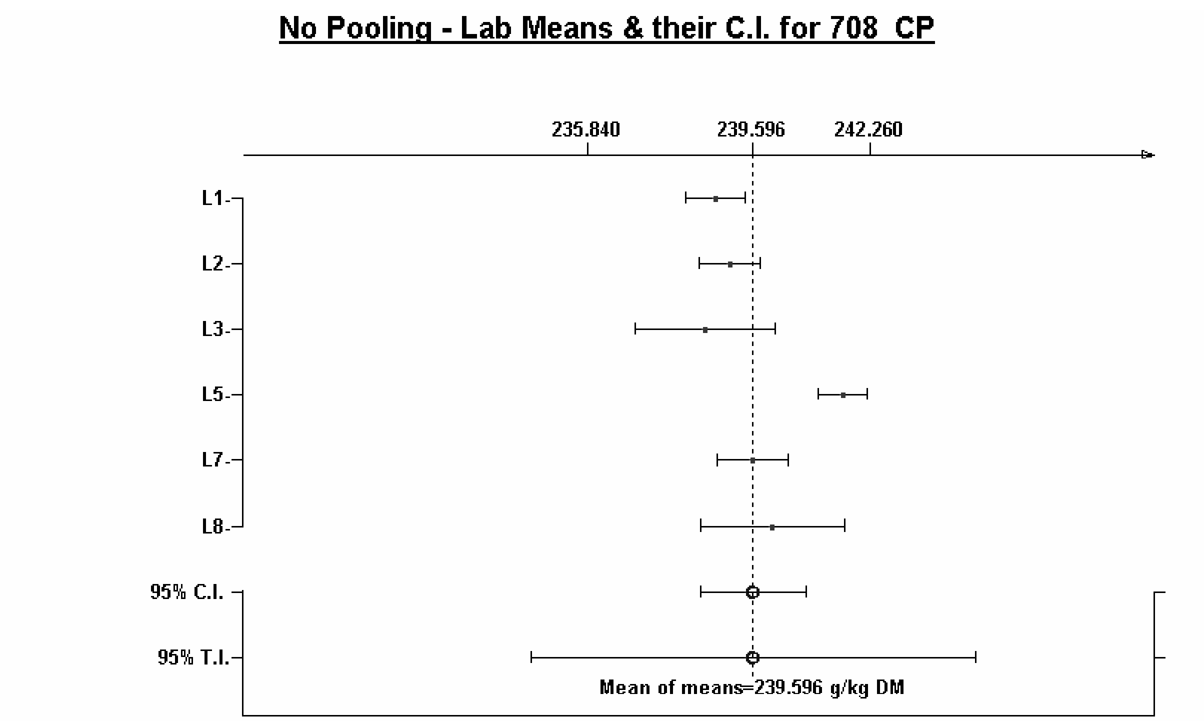

Figure 11.1 – Graphical presentation of results for BCR-708 Content of crude protein

Table 11.2 - BCR-708 Content of Crude oils and fats.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	65.767	0.652	0.684	66.040	66.290	66.550	65.700	64.930	65.090
1	66.422	1.555	1.632	64.300	65.240	67.640	66.000	66.840	68.510
2	62.833	1.259	1.321	63.360	63.100	64.250	60.760	61.970	63.560
3	63.507	1.024	1.074	62.400	62.120	63.560	64.300	64.560	64.100
4	64.317	0.754	0.792	63.580	63.290	64.190	65.040	65.020	64.780
7	66.817	0.806	0.846	66.100	66.800	67.900	66.100	67.700	66.300
8	62.958	1.057	1.313	64.080	61.970	61.780	63.090		63.870
			Range [min..max]						[60.760 .. 68.510]
			Mean of means						64.660
			95 % H.W. Confidence Interval						1.541

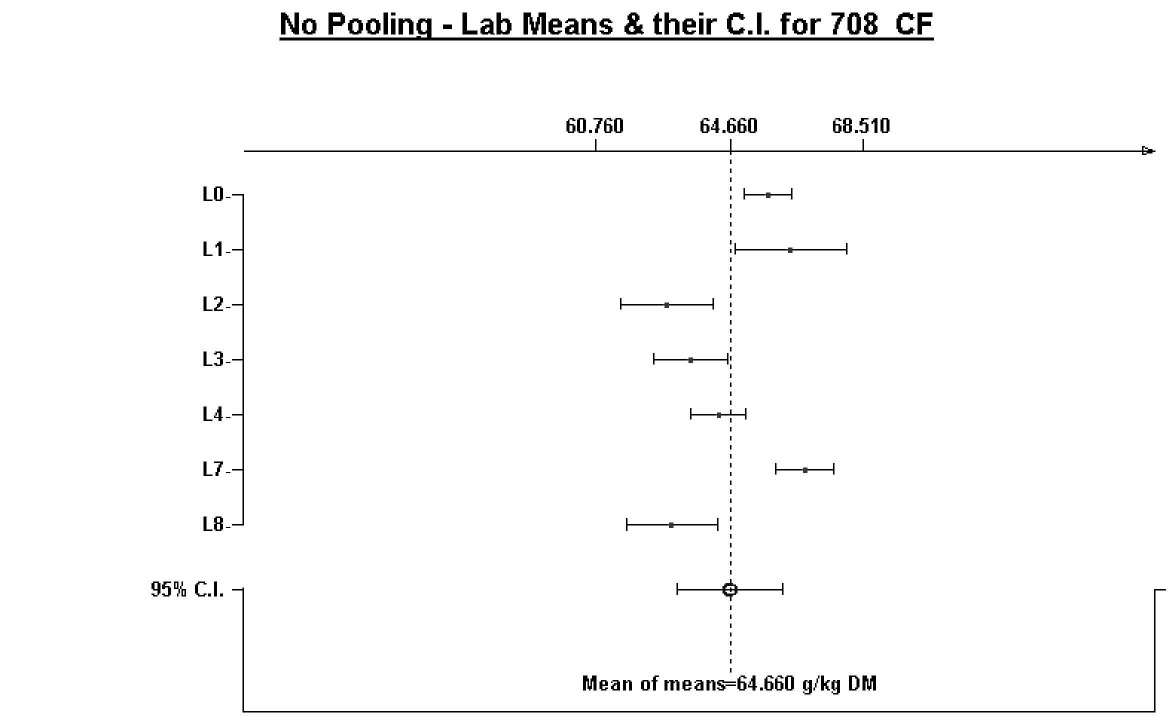

Figure 11.2 - Graphical presentation of results for BCR-708 Content of Crude oils and fats.

Table 11.3 – BCR-708 Content of crude fibre.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	91.462	1.041	1.093	92.810	92.590	91.080	91.240	90.090	90.960
1	92.163	0.313	0.328	91.960	92.170	92.070	91.950	92.050	92.780
2	99.630	0.472	0.495	100.240	99.040	100.150	99.320	99.530	99.500
3	87.128	1.262	1.324	87.980	86.630	87.540	88.810	85.200	86.610
4	93.248	1.124	1.179	93.720	93.870	93.980	93.070	93.800	91.050
5	95.245	2.063	2.165	98.950	94.250	96.450	93.940	93.930	93.950
6	90.112	1.580	1.658	89.260	92.010	88.000	90.010	89.470	91.920
7	96.633	1.124	1.179	96.300	95.300	96.500	98.300	97.600	95.800
8	94.055	1.519	1.594	94.540	95.590	95.290	91.480	93.190	94.240
Range [min..max]									[85.200 .. 100.240]
Mean of means									93.297
95 % H.W. Confidence Interval									2.830

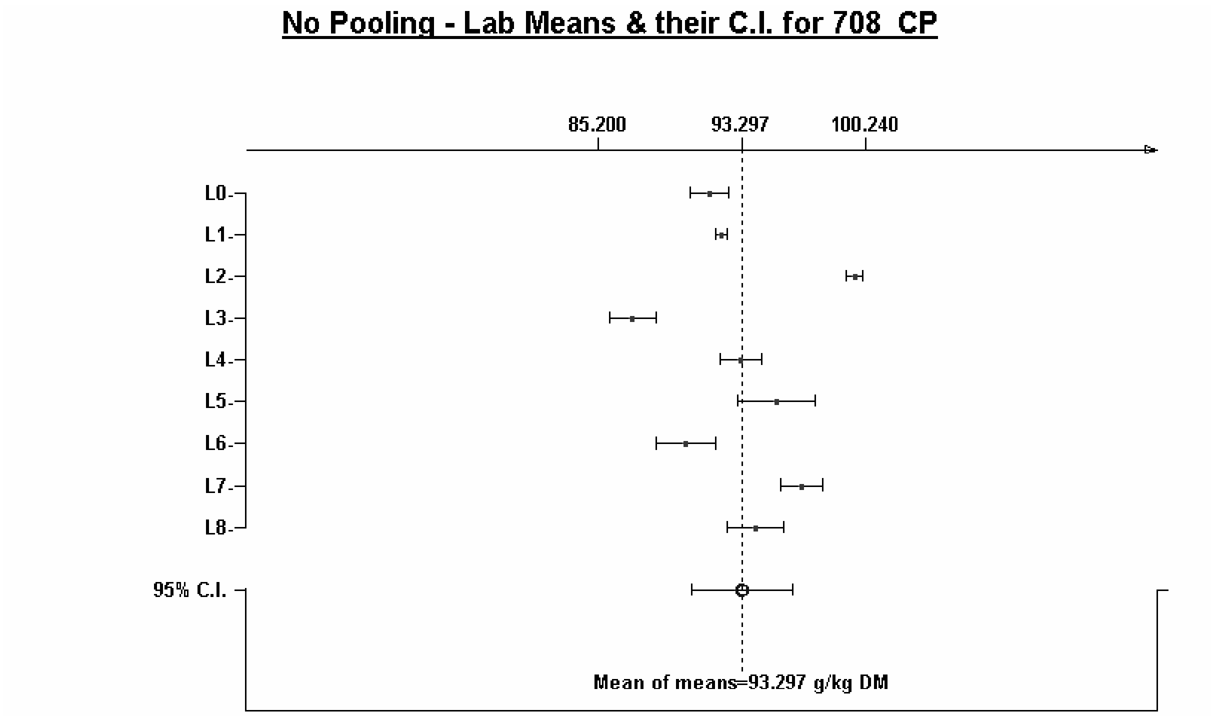

Figure 11.3 - Graphical presentation of results for BCR-708 Content of crude fibre.

Table 11.4 – BCR-708 Content of crude ash.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	50.092	0.240	0.252	50.290	50.070	50.010	49.720	50.410	50.050
1	50.470	0.055	0.057	50.420	50.420	50.420	50.520	50.520	50.520
2	50.168	0.245	0.257	49.740	50.070	50.420	50.320	50.150	50.310
3	49.867	0.659	0.692	50.440	50.490	50.470	49.220	49.360	49.220
4	49.568	0.157	0.164	49.480	49.330	49.510	49.690	49.750	49.650
6	50.020	0.432	0.537	49.500	50.600	50.300	49.800	49.900	
7	49.950	0.729	0.765	48.500	50.200	50.300	50.200	50.500	50.000
8	49.998	0.493	0.518	49.370	49.370	50.260	50.470	50.260	50.260
			Range [min..max]						[48.500 .. 50.600]
			Mean of means						50.017
			95 % H.W. Confidence Interval						0.215

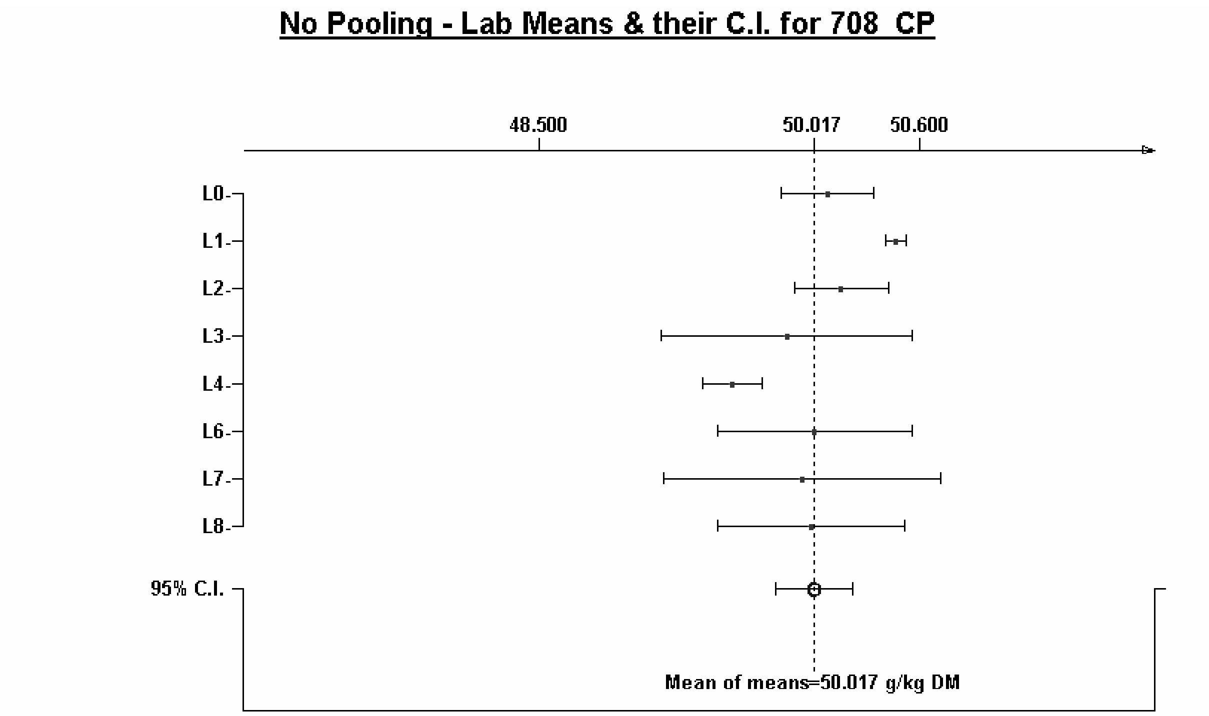

Figure 11.4 - Graphical presentation of results for BCR-708 Content of crude ash.

Table 11.5 – BCR-708 Content of copper.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	36.928	1.910	2.004	35.800	40.100	36.140	36.580	34.780	38.170
2	36.140	0.968	1.016	35.460	35.430	35.450	36.840	37.780	35.880
3	36.912	0.429	0.450	36.910	37.610	36.860	36.650	37.100	36.340
5	36.713	0.925	0.971	37.970	37.450	37.100	35.950	36.150	35.660
6									
7	36.938	2.244	2.355	34.370	36.450	37.090	34.840	40.280	38.600
8	39.185	1.296	1.361	39.920	37.820	37.700	41.010	38.870	39.790
			Range [min..max]						[34.370 .. 41.010]
			Mean of means						37.136
			95 % H.W. Confidence Interval						1.101

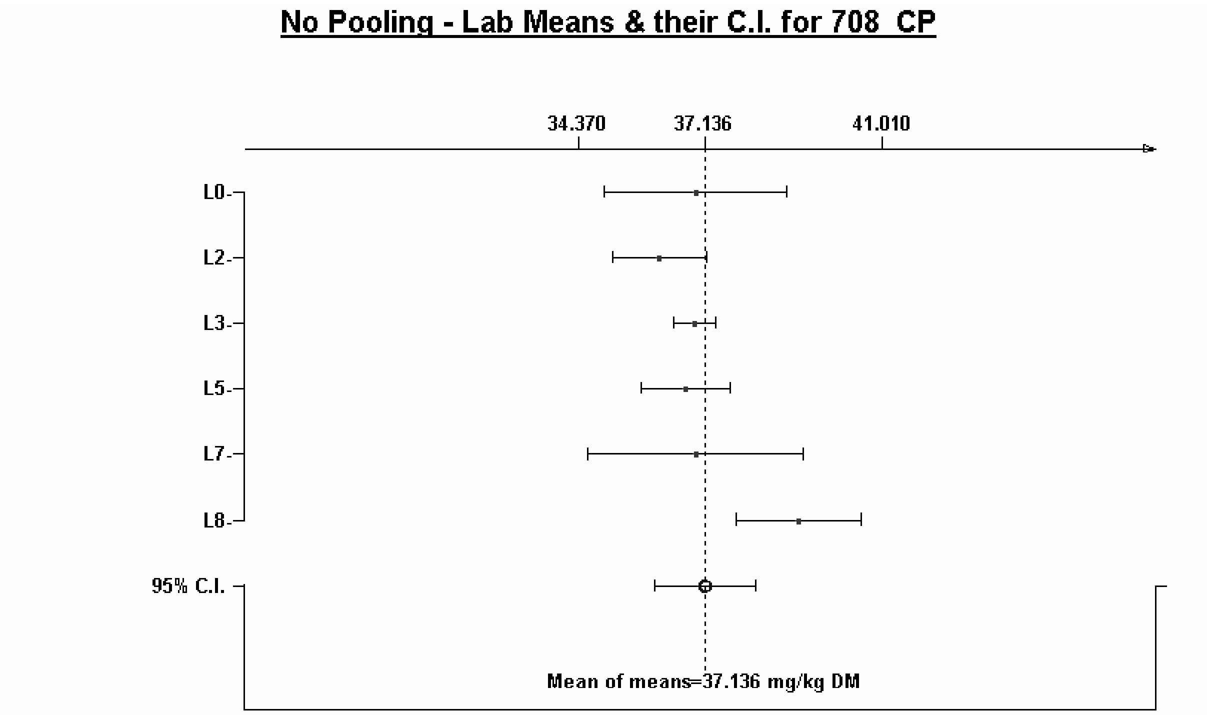

Figure 11.5 - Graphical presentation of results for BCR-708 Content of copper.

Table 11.6 – BCR-708 Content of calcium.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
1	4.725	0.060	0.063	4.760	4.710	4.800	4.730	4.620	4.730
2	4.923	0.122	0.128	4.700	4.990	5.010	5.000	4.860	4.980
3	4.828	0.032	0.033	4.830	4.850	4.860	4.840	4.820	4.770
4	4.720	0.090	0.095	4.590	4.660	4.820	4.820	4.710	4.720
5	4.773	0.084	0.088	4.870	4.670	4.870	4.780	4.750	4.700
7	4.785	0.120	0.126	4.820	4.590	4.960	4.780	4.750	4.810
8	4.812	0.108	0.114	4.830	4.940	4.820	4.840	4.830	4.610
			Range [min..max]						[4.590 .. 5.010]
			Mean of means						4.795
			95 % H.W. Confidence Interval						0.064

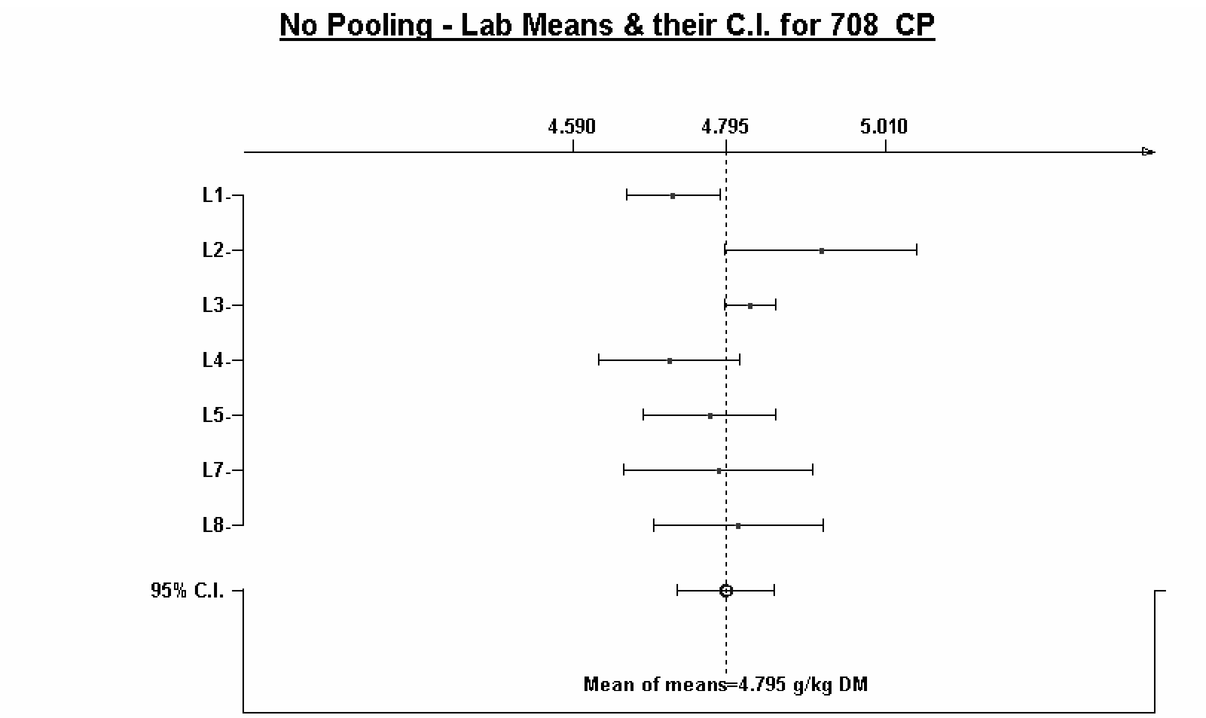

Figure 11.6 - Graphical presentation of results for BCR-708 Content of calcium.

Table 11.7 – BCR-708 Content of magnesium.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	1.523	0.043	0.045	1.470	1.490	1.500	1.570	1.570	1.540
1	1.455	0.005	0.006	1.450	1.450	1.460	1.450	1.460	1.460
2	1.522	0.017	0.018	1.510	1.540	1.530	1.510	1.540	1.500
3	1.413	0.044	0.046	1.370	1.380	1.370	1.450	1.460	1.450
4	1.408	0.031	0.033	1.390	1.440	1.450	1.410	1.370	1.390
5	1.507	0.025	0.026	1.520	1.460	1.530	1.520	1.510	1.500
7	1.492	0.081	0.085	1.580	1.410	1.580	1.530	1.420	1.430
8	1.470	0.070	0.073	1.470	1.580	1.360	1.470	1.470	1.470
			Range [min..max]						[1.360 .. 1.580]
			Mean of means						1.474
			95 % H.W. Confidence Interval						0.038

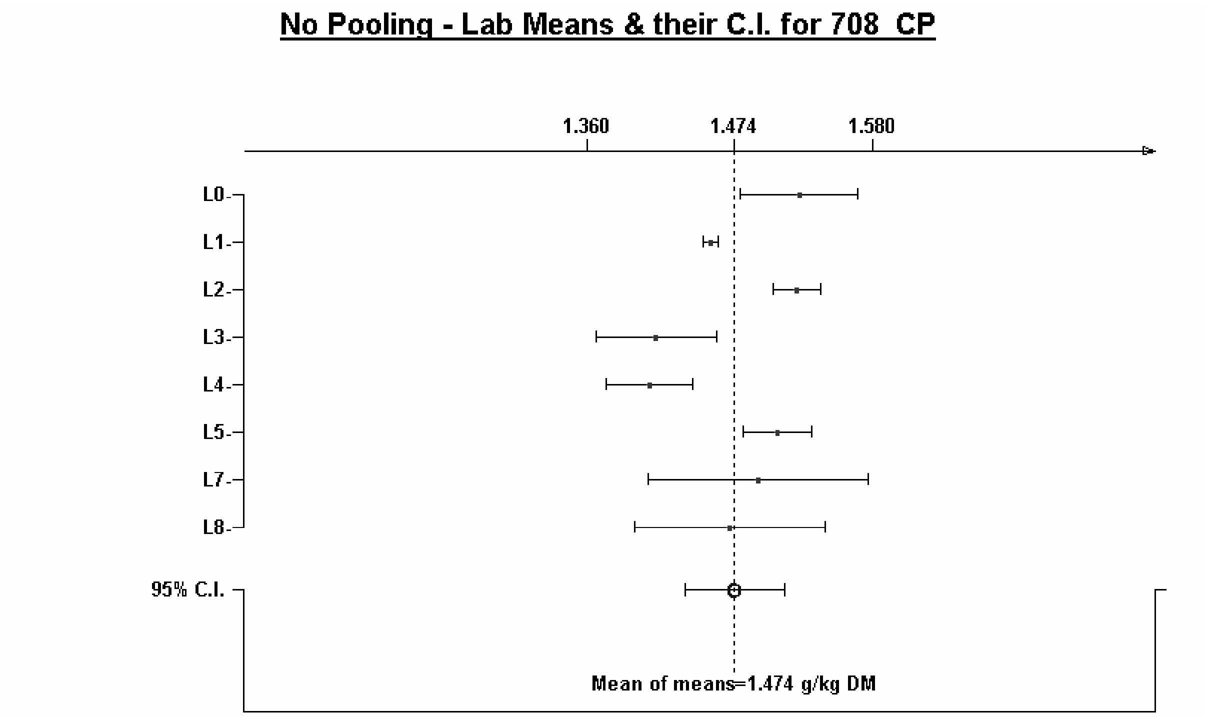

Figure 11.7 - Graphical presentation of results for BCR-708 Content of magnesium.

Table 11.8 – BCR-708 Content of phosphorus.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	4.805	0.131	0.138	4.590	4.760	4.760	4.930	4.940	4.850
3	4.782	0.035	0.037	4.720	4.810	4.800	4.760	4.810	4.790
4	4.817	0.046	0.048	4.820	4.760	4.770	4.880	4.850	4.820
5	4.703	0.118	0.124	4.650	4.520	4.870	4.780	4.700	4.700
7	4.707	0.033	0.035	4.680	4.680	4.770	4.700	4.700	4.710
8	4.672	0.150	0.158	4.620	4.730	4.400	4.840	4.730	4.710
			Range [min..max]						[4.400 .. 4.940]
			Mean of means						4.748
			95 % H.W. Confidence Interval						0.064

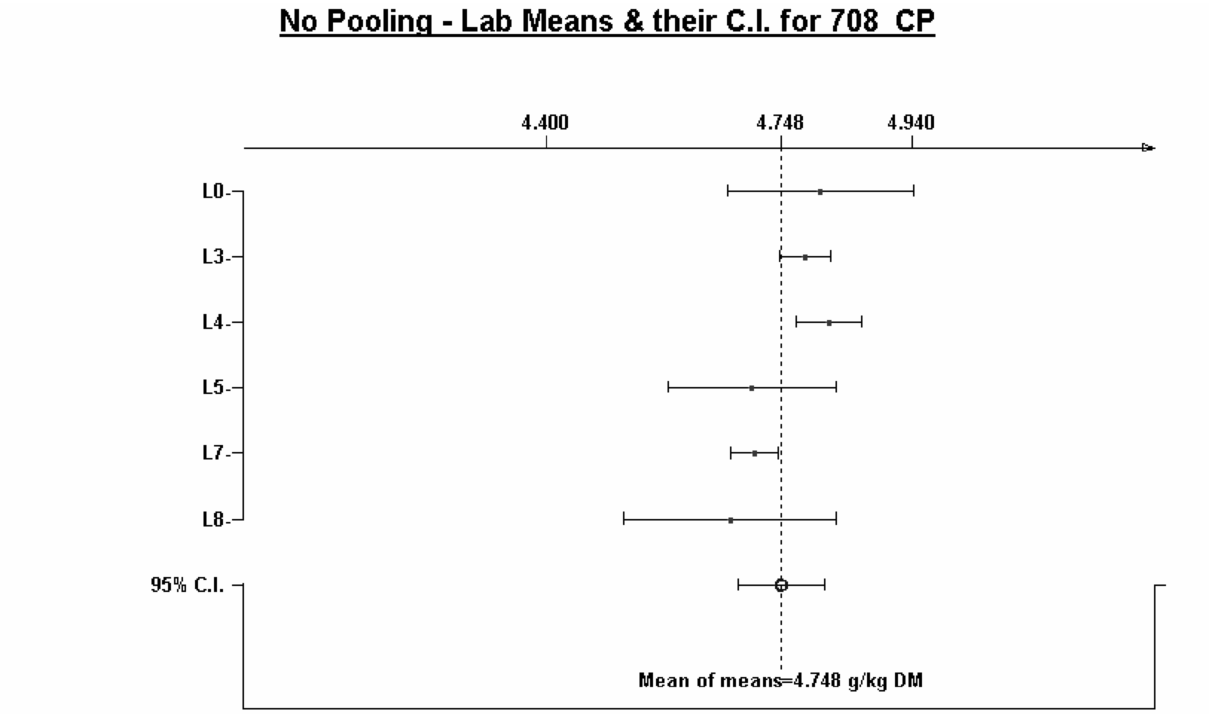

Figure 11.8 - Graphical presentation of results for BCR- BCR-708 Content of phosphorus.

Table 11.9 – BCR-709. Content of crude protein.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
1	199.898	0.427	0.448	199.580	199.690	200.530	200.110	200.110	199.370
2	197.613	0.301	0.315	197.190	197.360	197.810	197.790	197.550	197.980
3	198.640	0.979	1.027	198.650	198.860	197.320	200.250	198.010	198.750
4	196.025	1.917	2.012	196.020	196.740	198.000	197.150	195.770	192.470
5	202.022	1.034	1.085	202.640	203.170	201.790	200.210	202.530	201.790
7	198.980	0.512	0.538	199.157	199.684	198.853	199.190	198.861	198.137
8	198.343	0.954	1.001	199.370	199.360	198.510	198.300	197.470	197.050
			Range [min..max]						[192.470 .. 203.170]
			Mean of means						198.789
			95 % H.W. Confidence Interval						1.729

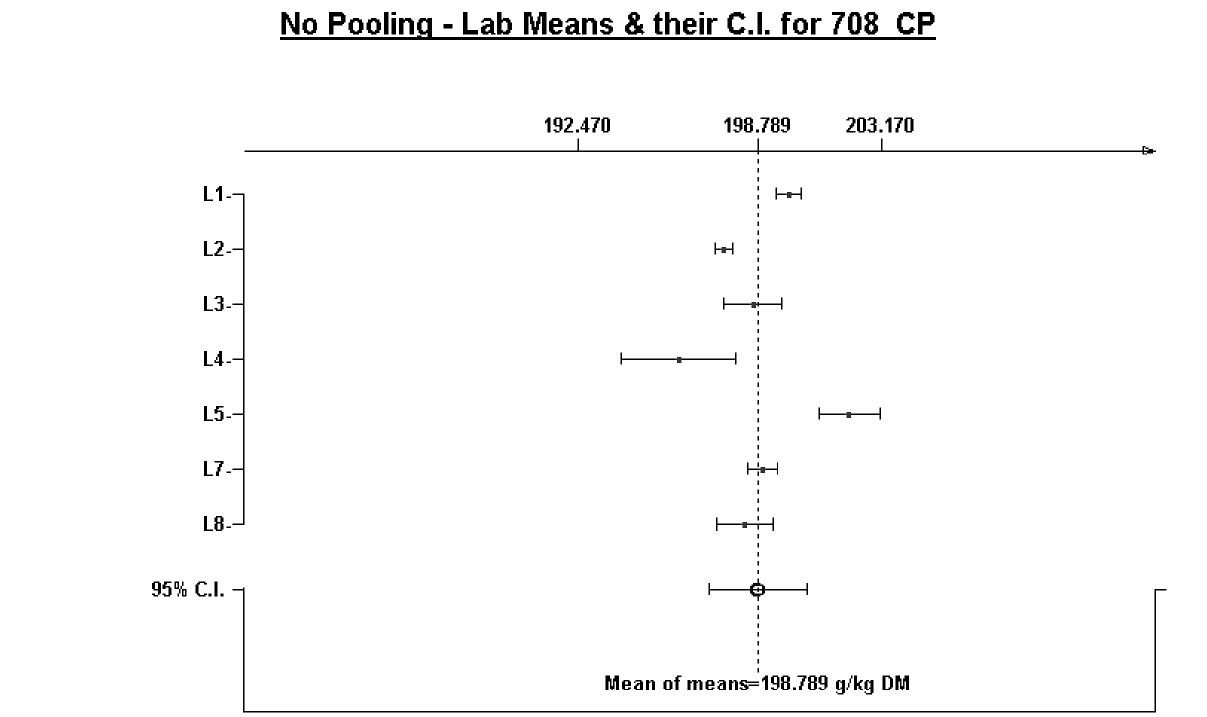

Figure 11.9 - Graphical presentation of results for BCR-709. Content of crude protein.

Table 11.10 – BCR-709. Content of crude oils and fats.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	51.413	1.270	1.332	52.960	52.820	51.590	50.760	49.890	50.460
1	48.467	1.539	1.615	50.160	50.370	48.580	47.320	46.580	47.790
3	49.510	0.617	0.647	48.710	49.050	49.250	49.640	50.220	50.190
4	51.403	0.648	0.680	50.640	50.900	52.420	51.470	51.170	51.820
5	50.675	0.435	0.457	49.840	50.580	50.950	50.890	50.790	51.000
7	52.034	0.648	0.680	51.781	53.045	51.862	51.500	51.417	52.597
			Range [min..max]						[46.580 .. 53.045]
			Mean of means						50.584
			95 % H.W. Confidence Interval						1.416

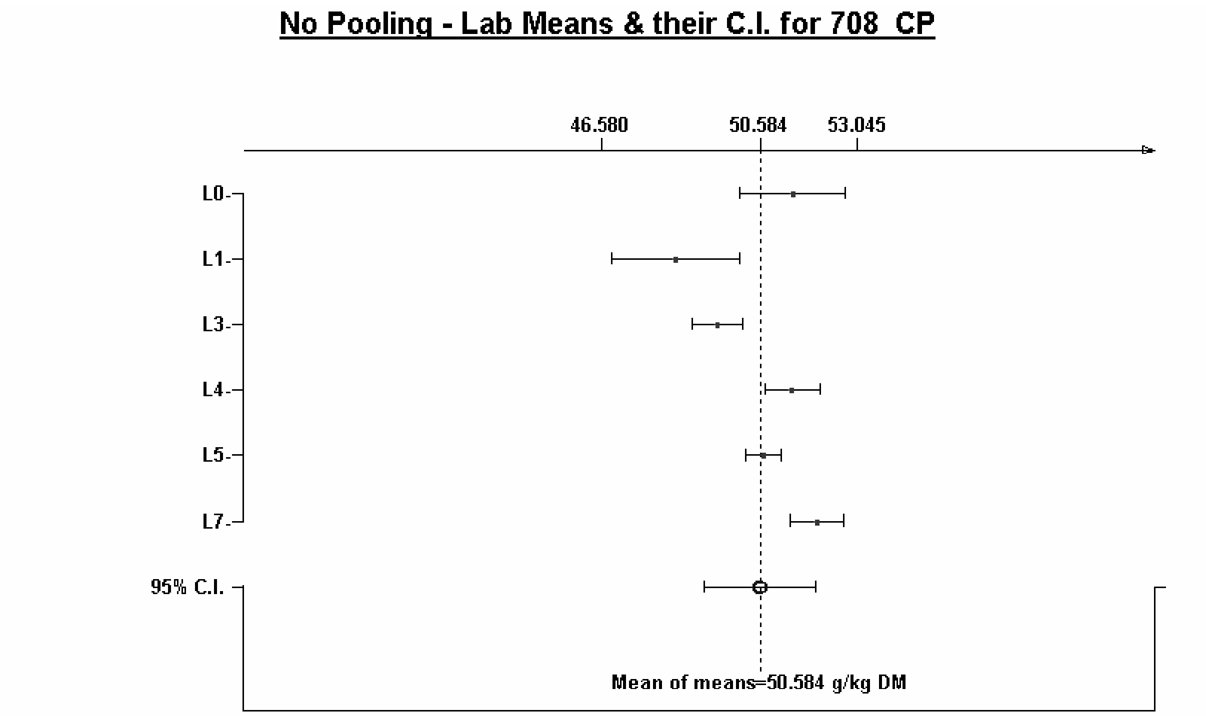

Figure 11.10 - Graphical presentation of results for BCR-709. Content of crude oils and fats.

Table 11.11 – BCR-709. Content of crude fibre.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
1	54,010	1,404	1,473	54,760	54,090	56,360	52,460	53,070	53,320
2	56,455	0,391	0,410	56,570	56,770	55,840	56,260	56,360	56,930
3	57,897	1,011	1,061	56,800	57,670	57,380	59,190	57,230	59,110
4	50,425	1,472	1,545	52,300	51,590	51,050	48,370	49,790	49,450
5	56,135	0,524	0,550	55,840	56,730	56,620	55,370	55,890	56,360
6	56,538	1,248	1,310	57,970	56,710	55,800	56,360	57,760	54,630
7	56,513	1,186	1,244	57,010	57,280	54,670	57,070	55,410	57,640
8	56,594	0,978	1,026	56,660	55,600	56,005	57,327	58,125	55,846
9	55,532	1,326	1,391	56,960	54,080	54,140	56,200	54,910	56,900
			Range [min..max]						[48,370 .. 59,190]
			Mean of means						55,567
			95 % H.W. Confidence Interval						1,682

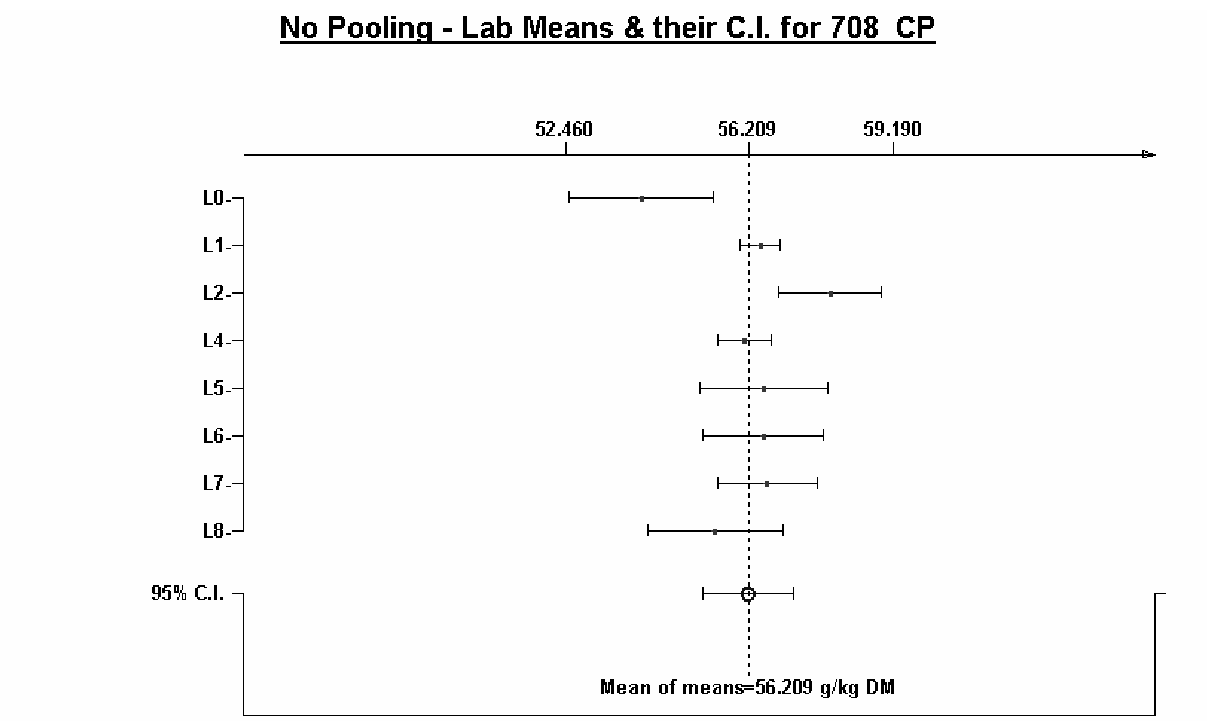

Figure 11.11 - Graphical presentation of results for BCR-709. Content of crude fibre

Table 11.12 – BCR-709. Content of crude ash.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	42.025	0.386	0.405	41.940	42.320	42.270	41.630	42.460	41.530
1	42.093	0.295	0.310	42.270	42.290	42.480	41.750	41.960	41.810
2	42.287	0.222	0.233	42.330	42.340	42.270	42.050	42.660	42.070
3	41.727	0.439	0.461	41.530	42.390	42.170	41.510	41.340	41.420
4	41.850	0.121	0.127	41.870	41.950	42.030	41.760	41.740	41.750
5	41.113	0.220	0.231	41.290	41.180	41.350	41.110	41.010	40.740
6	41.820	0.259	0.321	41.900	41.700	41.800	42.200	41.500	
7	41.792	0.206	0.216	42.044	41.926	41.923	41.685	41.682	41.492
8	41.608	0.482	0.506	42.190	41.360	41.400	41.360	42.240	41.100
			Range [min..max]						[40.740 .. 42.660]
			Mean of means						41.813
			95 % H.W. Confidence Interval						0.256

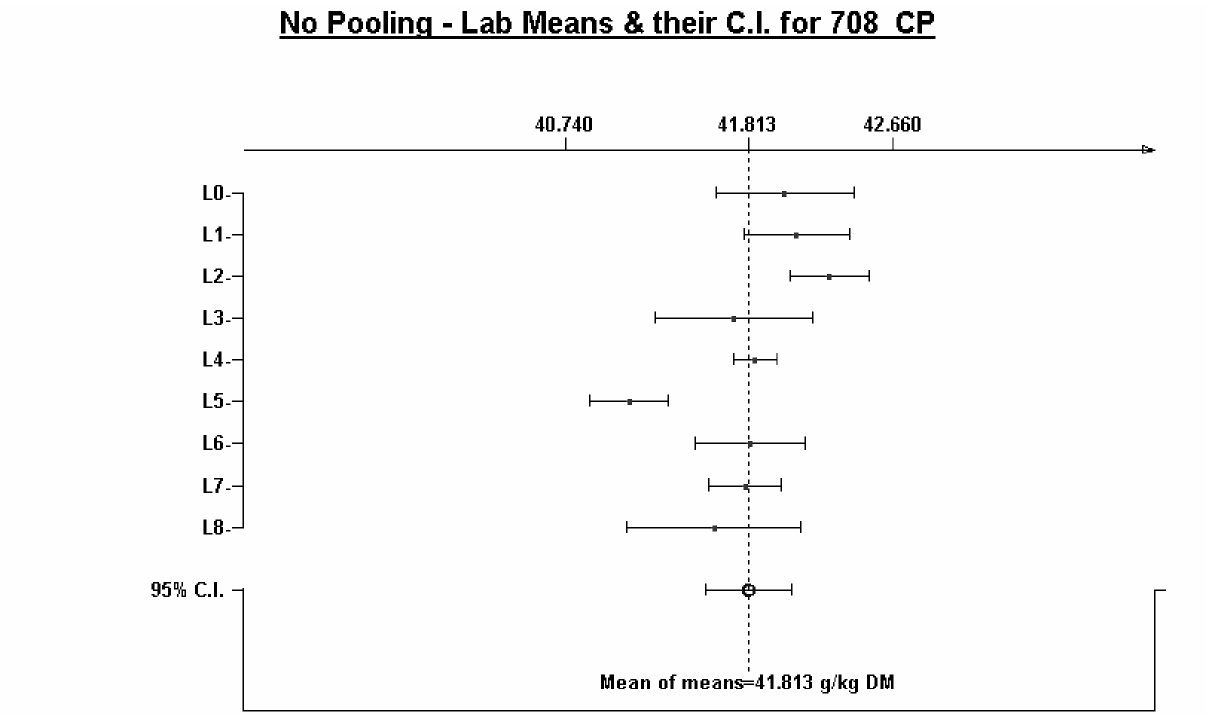

Figure 11.12 - Graphical presentation of results for BCR-709. Content of crude ash.

Table 11.13 – BCR-709. Content of copper.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
1	169.908	3.273	3.434	172.450	165.790	172.450	173.500	167.190	168.070
2	182.003	6.240	6.548	183.800	183.670	178.940	175.610	192.760	177.240
3	169.790	1.853	1.945	168.850	171.900	172.060	169.940	167.670	168.320
5	175.745	2.541	2.666	174.230	176.350	178.270	178.760	174.740	172.120
7	172.350	3.846	4.036	168.350	172.630	167.020	176.130	175.790	174.180
8	169.933	3.216	3.375	165.610	166.490	171.970	172.850	169.830	172.850
Range [min..max]									[165.610 .. 192.760]
Mean of means									173.288
95 % H.W. Confidence Interval									5.094

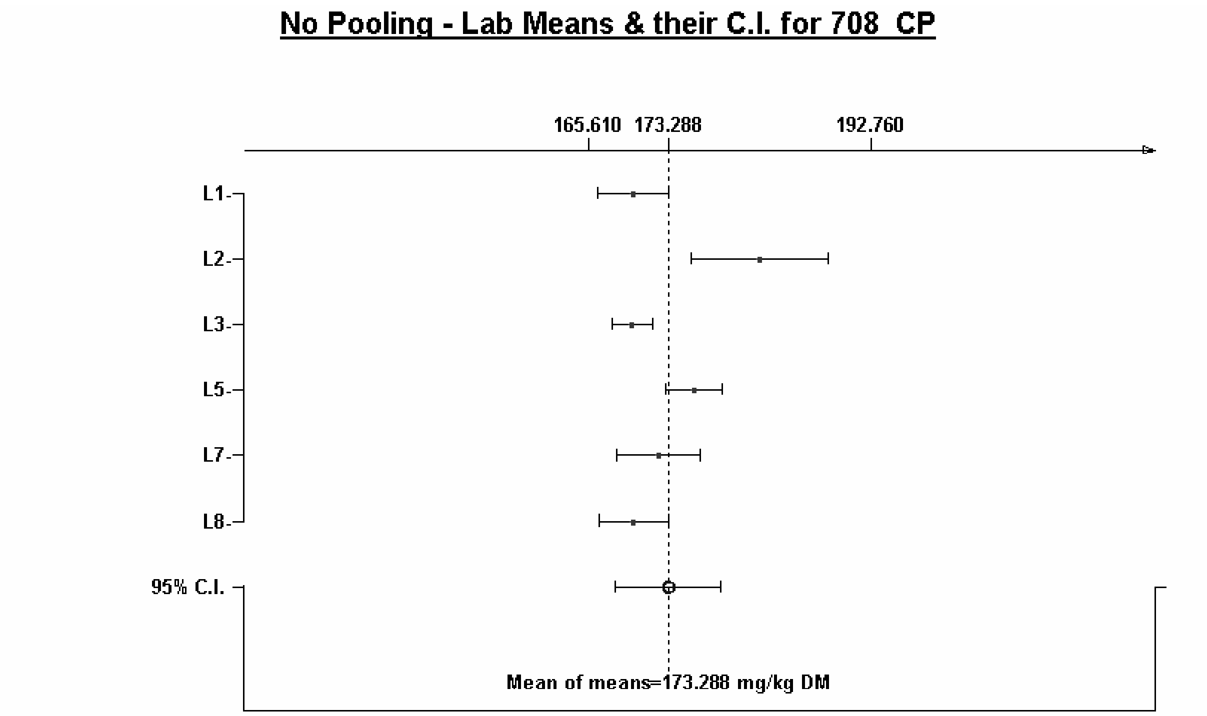

Figure 11.13 - Graphical presentation of results for BCR-709. Content of copper.

Table 11.14 – BCR-709. Content of calcium.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	1.173	0.035	0.037	1.190	1.130	1.230	1.150	1.180	1.160
1	1.068	0.037	0.038	1.110	1.060	1.050	1.010	1.100	1.080
2	1.088	0.045	0.047	1.130	1.050	1.110	1.070	1.140	1.030
3	1.001	0.021	0.022	1.040	0.995	0.990	0.980	1.000	1.000
4	0.918	0.077	0.081	0.970	0.980	1.000	0.810	0.850	0.900
7	1.062	0.044	0.046	1.120	1.030	1.050	1.080	1.090	1.000
8	1.045	0.059	0.062	1.160	1.010	1.000	1.030	1.050	1.020
			Range [min..max]						[0.810 .. 1.230]
			Mean of means						1.051
			95 % H.W. Confidence Interval						0.073

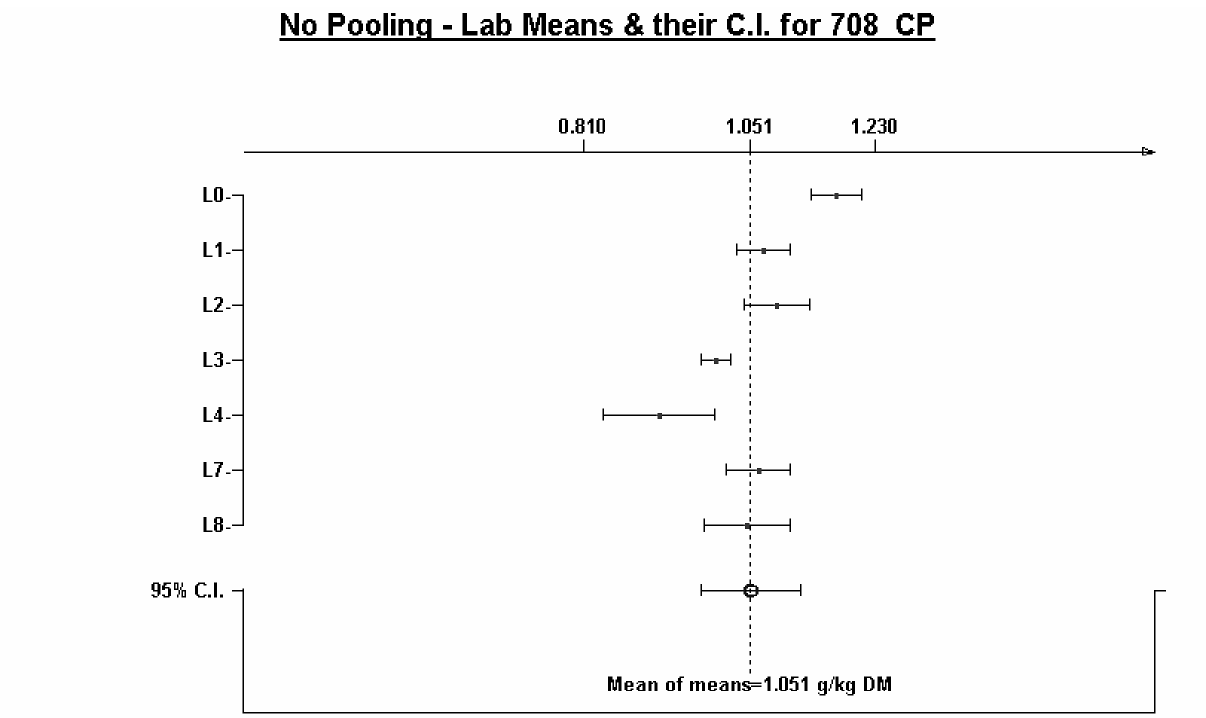

Figure 11.14 - Graphical presentation of results for BCR-709. Content of calcium.

Table 11.15 – BCR-709. Content of magnesium.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	1.980	0.040	0.042	1.950	1.930	1.990	2.020	1.960	2.030
1	1.838	0.052	0.055	1.760	1.800	1.860	1.900	1.830	1.880
2	1.953	0.020	0.021	1.950	1.950	1.930	1.950	1.990	1.950
3	1.852	0.041	0.043	1.800	1.810	1.840	1.890	1.880	1.890
4	1.762	0.031	0.033	1.750	1.810	1.730	1.770	1.730	1.780
5	1.945	0.008	0.009	1.940	1.940	1.940	1.960	1.950	1.940
8	1.888	0.048	0.051	1.790	1.910	1.910	1.910	1.900	1.910
			Range [min..max]	[1.730 .. 2.030]					
			Mean of means	1.888					
			95 % H.W. Confidence Interval	0.071					

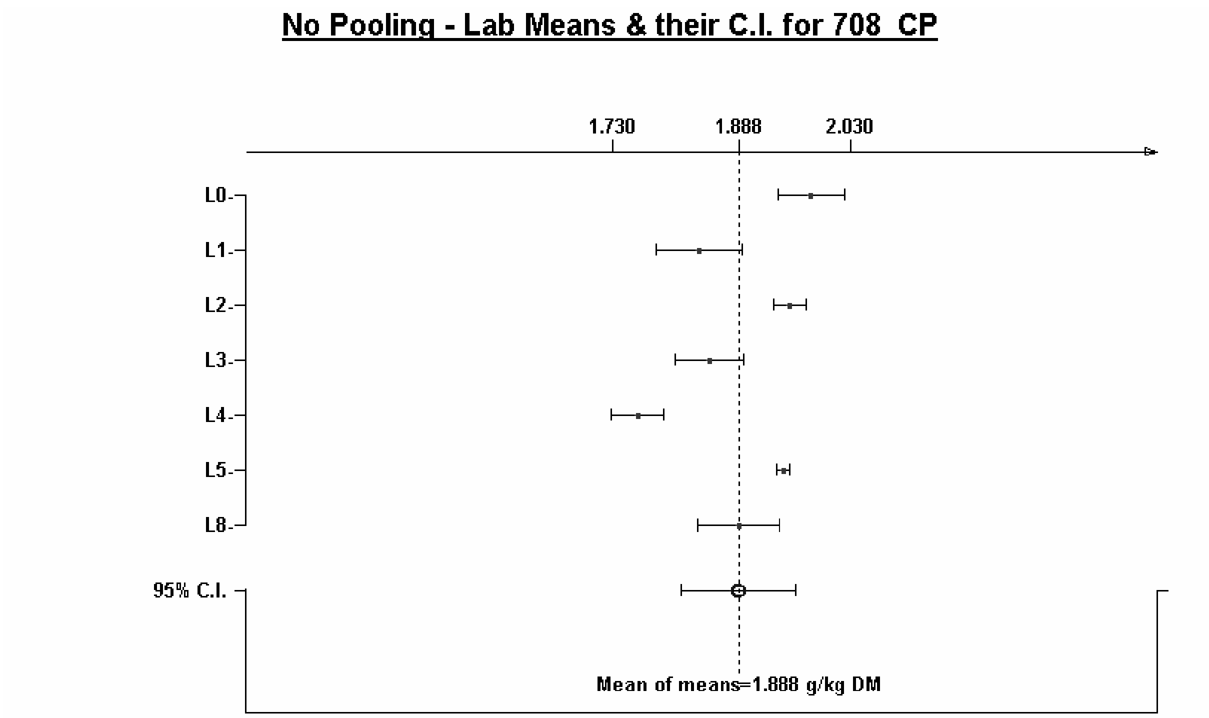

Figure 11.15 - Graphical presentation of results for BCR-709. Content of magnesium.

Table 11.16 – BCR-709. Content of phosphorus.

Lab	*Mean*	*Std. Dev.*	*H.W. CI (95 %)*	*Sample #1*	*Sample #2*	*Sample #3*	*Sample #4*	*Sample #5*	*Sample #6*
0	5.485	0.072	0.075	5.390	5.460	5.470	5.580	5.450	5.560
1	5.348	0.208	0.218	4.990	5.330	5.260	5.570	5.470	5.470
2	5.460	0.076	0.079	5.550	5.510	5.380	5.500	5.460	5.360
5	5.392	0.029	0.031	5.350	5.440	5.390	5.390	5.400	5.380
7	5.387	0.056	0.058	5.370	5.280	5.420	5.410	5.420	5.420
8	5.398	0.077	0.081	5.270	5.410	5.410	5.510	5.380	5.410
			Range [min..max]						[4.990 .. 5.580]
			Mean of means						5.412
			95 % H.W. Confidence Interval						0.053

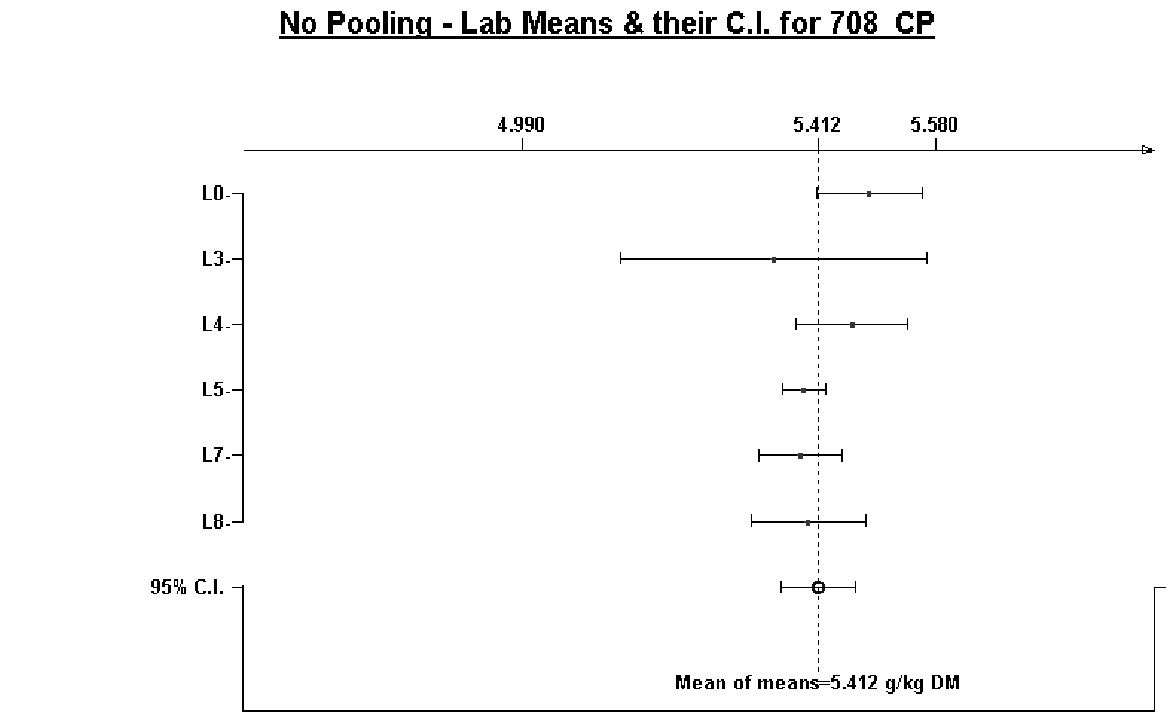

Figure 11.16 - Graphical presentation of results for BCR-709. Content of phosphorus.

12. ANNEX III – RESULTS OF STABILITY STUDIES

Table 12.1 - Results of the stability study for BCR-708. Ratios R_T of mean values (R_T=XT/Xref) at +4 °C, +20 °C, +40 °C, and +70 °C versus -20 °C.

Crude protein		*Slope ≠ 0*	*Weeks* 2	*Weeks* 4	*Weeks* 6	*Weeks* 8	*Weeks* 52
	R(4)	No	1.007	1.003	1.001	1.022	0.995
	R(20)	No	1.026	0.995	0.998	1.006	0.997
	R(40)	No	1.006	0.991	1.002	1.010	
	R(70)	No	1.023	1.023	1.022	1.028	
Crude Fats and Oils	R(4)	Yes (p>0.95) No (p>0.99)	1.020	0.993	0.970	1.011	0.944
	R(20)	No	0.982	0.983	0.973	1.011	0.977
	R(40)	No	0.979	0.944	0.939	0.963	
	R(70)	No	0.968	0.965	1.001	0.893	
Crude fibre	R(4)	No	0.978	0.998	1.015	0.997	1.023
	R(20)	Yes (p<0.99)	0.987	1.010	1.015	0.983	1.083
	R(40)	No	0.938	0.997	1.019	0.949	
	R(70)	No	0.938	0.986	1.031	1.023	
Crude Ash	R(4)	No	0.999	1.011	1.007	0.999	0.994
	R(20)	No	1.001	1.014	1.008	1.005	0.997
	R(40)	No	1.003	1.016	1.002	1.001	
	R(70)	No	1.011	1.036	1.029	1.014	
Calcium	R(4)	No	0.980	1.000	0.989	0.950	0.984
	R(20)	No	0.989	1.001	0.951	0.960	0.984
	R(40)	No	0.989	0.974	1.052	0.970	
	R(70)	No	0.957	0.991	1.048	0.998	
Phosphorus	R(4)	No	1.000	0.989	0.987	0.955	0.981
	R(20)	No	0.988	0.990	0.958	0.978	0.990
	R(40)	No	0.988	0.963	1.041	1.012	
	R(70)	No	0.952	0.990	1.043	1.020	
Magnesium	R(4)	No	1.000	1.011	0.996	0.963	0.983
	R(20)	No	0.999	1.004	0.908	0.970	0.999
	R(40)	No	0.999	0.988	1.085	1.000	
	R(70)	No	0.971	1.008	1.069	1.005	

Table 12.2 - Results of the stability study for BCR-709. Ratios R_T of mean values (R_T=XT/Xref) at +4 °C, +20 °C, +40 °C, and +70 °C versus -20 °C.

		Slope ≠ 0	*Weeks 2*	*Weeks 4*	*Weeks 6*	*Weeks 8*	*Weeks 52*
Crude protein	R(4)	No	1.001	0.992	0.999	1.001	1.003
	R(20)	No	1.002	0.993	0.996	1.005	1.005
	R(40)	No	1.003	0.988	0.993	1.014	
	R(70)	No	1.006	0.997	0.994	1.012	
Crude Fats and Oils	R(4)	Yes (p>0.95)	0.921	0.990	1.000	0.945	0.800
	R(20)	No	0.910	0.971	0.968	0.930	0.934
	R(40)	No	0.902	0.945	0.913	0.905	
	R(70)	No	0.898	0.937	0.914	0.905	
Crude Fibre	R(4)	No	1.058	0.977	0.982	0.980	0.988
	R(20)	No	1.055	0.967	0.976	0.995	1.089
	R(40)	No	1.027	0.992	0.986	1.058	
	R(70)	No	0.985	1.012	1.047	1.026	
Crude Ash	R(4)	No	1.009	1.017	1.006	0.994	0.999
	R(20)	No	1.012	1.036	0.998	1.005	0.999
	R(40)	No	1.011	1.013	1.000	1.000	
	R(70)	No	1.018	1.016	1.009	1.005	
Calcium	R(4)	No	0.954	1.012	1.024	1.000	0.981
	R(20)	No	1.001	1.021	0.982	1.050	0.998
	R(40)	No	1.000	1.003	0.986	1.035	
	R(70)	No	1.001	1.030	0.998	1.054	
Phosphorus	R(4)	No	1.000	0.986	1.037	0.959	0.979
	R(20)	No	0.992	0.979	1.022	0.980	0.998
	R(40)	No	0.991	0.963	1.046	0.980	
	R(70)	No	0.982	0.992	1.025	1.004	
Magnesium	R(4)	No	0.947	0.980	0.998	0.972	0.983
	R(20)	No	0.949	0.983	1.004	1.056	0.989
	R(40)	No	0.974	0.969	1.046	1.000	
	R(70)	No	0.975	1.002	1.022	1.004	

European Commission

EUR 21070 – DG Joint Research Centre, Institute for Reference Materials and Measurements – Certification of the mass fractions of crude protein, crude oils and fats, crude fibre, crude ash and phosphorus (according to method specification laid down in EU-legislation) and of copper, calcium and magnesium, BCR -708 (synthetic dairy feed), BCR-709 (synthetic feed for growing pigs)
Authors, G.N. Kramer, B.M. Gawlik, A. Bernreuther, A. Lamberty, D. Bennink, E. Nordkvist
Luxembourg: Office for Official Publications of the European Communities
2004 – 44 pp. – 21.0 x 29.7 cm
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Abstract

This report describes the preparation, homogeneity and stability studies, and the certification of nutrients analysed in the official control of nutrients in feeding stuffs. The following analytes have been studied: Crude protein (N·6.25), Crude oils and fats, Crude fibre, Crude ash, Copper, Calcium, Phosphorus and Magnesium. For the analyses comprising the “proximate analysis scheme”, Crude protein, Crude oils and fats, Crude fibre and Crude ash, the studies aimed to method specific certification, according to the specifications laid down in EU-Directives. The elements on the other hand were analysed by different methods in order to produce an un-biased estimate of their respective mass fraction in the materials. Two materials have been prepared, one is a synthetic feed for dairy cows (BCR-708) and one is a synthetic feed for growing pigs (BCR-709). The certified values and their associated uncertainties are given in the tables below. Expanded uncertainties (coverage factor k=2) were expressed according to the Guide for the Expression of Uncertainties in Measurement (GUM [6]).

Certified values of mass fractions of nutrients in BCR-708 and BCR-709

Material	*BCR-708 synthetic dairy feed*	*BCR-709 synthetic pig feed*
Analyte	Mass fraction ± Uncertainty*	Mass fraction ± Uncertainty*
Crude Protein (N·6.25)	240 ± 12 g/kg	199 ± 5 g/kg
Crude Oils & Fats	65 ± 8 g/kg	51 ± 14 g/kg
Crude Fibre	93 ± 14 g/kg	56 ± 12 g/kg
Crude Ash	50.0 ± 3.0 g/kg	42 ± 4 g/kg
Calcium	4.8 ± 0.5 g/kg	1.05 ± 0.16 g/kg
Copper	37 ± 4 mg/kg	173 ± 25 mg/kg
Magnesium	1.47 ± 0.22 g/kg	1.89 ± 0.30 g/kg
Phosphorus	4.7 ± 0.4 g/kg	5.4 ± 0.7 g/kg

*Expressed as expanded uncertainties with a coverage factor k=2 according to the GUM.

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